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ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE

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INTRODUCTION

An account of the placentae and uteri at a relatively late stage of gestation of four specimens of Père David's deer (*Elaphurus davidianus*) has already been given by two of us (Harrison & Hamilton, 1952). The appearance and microscopical structure of the placentomes and the remarkable changes found in certain zones of the villi prompted us to look further into the whole question of placentation in other Cervidae which became available.

Brief and often incomplete accounts have been given of the placentae of a number of deer by earlier investigators. Weber, as early as 1832, and Bischoff (1854) described the form, and to some extent the structure, of the placenta of the roe deer (*Capreolus capreolus*). Swinhoe (1870) gave an account of reproduction of the Shanghai river deer (Chinese water deer) (*Hydropotes inermis*) and some details of the placenta of this deer were also given by Ewart (1878). Turner (1878*b*) described the gravid uterus and foetal membranes of the hog-deer (*Cervus porcinus*). The amniotic cavity had been opened before it came into Turner's hands and only one of the 'foetal caruncles' was attached to the 'maternal cotyledon'. In the same year Turner (1878*a*) gave an account of the foetal membranes after they had been shed by the reindeer (*Rangifer tarandus*). In 1879 Turner described the arrangement of the foetal membranes in the uterus of the Mexican deer (*Cervus mexicanus*).

Beauregard & Boulart (1885), in their note on the placentation of ruminants, referred to the number of 'cotyledons' among the following deer: *Panolia frontalis*, *Cervus porcinus*, *Cervus elaphus* and *Rangifer tarandus*. These authors were more concerned with the phylogenetic relationship of deer to other ruminants than with a description of the placenta. A brief note on the placenta of *Cervus sica* was given by the same authors in 1885. Boulart (1888) briefly referred to the placenta of the Mexican deer (*Cervus mexicanus*). Strahl (1906) and Andresen (1922) have described the placenta of several specimens of the red deer (*Cervus elaphus*) and Kolster (1909) has described that of *Rangifer tarandus* in some detail. Brief descriptions of placental details in other Cervidae are included in a valuable review by Andresen (1927). Harrison & Hyett (1954) have given an account of the growth, changes in form and the gross structure of the foetal and maternal elements of the placentome of the fallow deer (*Dama dama*) from the time of development of cotyledonary villi until the foetus reached a C.R. length of 40 cm.

MATERIALS AND METHODS

Specimens of intact pregnant uteri or portions of the uterus and placenta from eight species of Cervidae have been available for examination (see Table 1 for details). Pregnant uteri of Père David's deer (*Elaphurus davidianus*) were obtained from

Woburn Park, Bedford, through the kindness of the late Duke of Bedford and have already been described (Harrison & Hamilton, 1952). Through the kindness of the late Lord Leconfield, who had a large herd of fallow deer (*Dama dama*) at Petworth, we have been able to obtain stages of development from the unimplanted chorionic sac to late in gestation. Additional specimens of this deer have been obtained from Parham Park, Sussex, through the kindness of the Hon. Clive Pearson, from Knole Park, Kent, through the kindness of Lord Sackville, and from Quendon Park, Essex, through the kindness of Sir Arthur Ellis. Over two hundred uteri at different stages of pregnancy, and some twenty non-pregnant uteri form the basis of our collection. The details of the pregnant series are given in Table 1, but lack of space prevents a detailed tabulation of the dates; these and other details are available from the authors. Some aspects of development and growth of the placentomes from these animals have been described by Harrison & Hyett (1954).

The pregnant specimens from the other species form only a small and incomplete series, but are included here in order to provide a comparison of placentation in the Cervidae at least at certain stages of pregnancy. The red deer (*Cervus elaphus*) were obtained from Richmond Park, Surrey, through the kindness of the local authorities. The roe deer (*Capreolus capreolus*) were obtained from Petworth Park, Sussex, the Manchurian Sika (*Capreolus bedfordi*) and Indian spotted deer (*Cervus axis*) from Woburn Park, Bedfordshire, and the Japanese Sika (*Cervus nippon*) from Knole Park, Kent. The specimens of pregnant uteri from the reindeer (*Rangifer tarandus*) were flown by air from Norway either as fixed or frozen specimens. They were supplied through the kindness of Dr O. W. Tenjord, of the Norwegian Legation, London. The specimens were collected by District Veterinary Surgeons at Alta and Karasjok, Norway. The gross details are satisfactory but the microscopical appearances are poor.

The material was dealt with in three ways, depending on its freshness. The most recently killed specimens of pregnant uteri were opened and portions of the ovaries, uterus and placenta were removed and fixed in a variety of fixatives, including formaldehyde, formol-saline, Bouin's fluid, Zenker-formol, 80 % alcohol or acetone (chilled) and Rossman's fluid (chilled). Representative blocks of tissue were subsequently embedded in paraffin and sections were prepared at 3–10 μ. Small pieces of placentome (foetuses of 22 mm. and 200 mm. C.R. length) were fixed in Palade's or Dalton's fluid, embedded in methacrylate and ultra-thin sections were examined in a Siemens Elmiskop I electron microscope.

Sections were stained with Heidenhain's iron haematoxylin, Masson's trichrome (Wislocki's modification), Mallory's stain and Robb-Smith's modification of Foot's method for impregnation of reticulum. Frozen sections were stained with Sudan Black B. McManus' (1946) periodic acid-Schiff procedure was employed on the material fixed in Rossman's fluid. Phosphatases, alkaline and acid, were demonstrated in tissue fixed in chilled acetone or 80 % alcohol (Dempsey & Deane, 1946). Preparations were also made for examination by the phase contrast microscope.

The second method of examination involved the selection of suitable, undamaged pregnant uteri and their injection with coloured gelatin, Marco Resin or Latex. Warm 20 % gelatin containing Boston Chemical Company's Dispersion Colours (10 %) was the most convenient injection mass, because it entered the finest capillaries and also allowed subsequent sectioning. Both maternal and foetal circulation were injected, or alternatively only one of them. These specimens were subsequently fixed in 10 % formalin.

Table 1. *Details of embryos and dates of collection*

Species	C.R. length in mm.	No. collected	Dates recovered
Fallow deer (*Dama dama*)	1·0	1	2 Dec.
	7·0–9·0	4	25 Nov.–2 Dec.
	12·0–17·0	8	25 Nov.–2 Dec. (1 on 12 Jan.)
	18·0–26·0	21	25 Nov.–18 Dec. (1 on 24 Feb.)
	27·0–40·0	8	8 Dec.–29 Jan.
	78·0–95·0	11	17 Dec.–29 Jan. (1 on 17 Feb.)
	99·0–110·0	31	7 Jan.–17 Feb. (1 on 2 Dec.; 1 on 23 Feb.)
	111·0–125·0	23	8–20 Jan.
	130·0–150·0	49	14 Jan.–10 Feb.
	152·0–170·0	24	25 Jan.–23 Feb.
	175·0–192·0	29	20 Jan.–26 Feb.
	195·0–220·0	21	10 Feb.–3 March (1 on 17 Jan.)
	222·0–290·0	27	17 Feb.–3 March
	375·0–399·0	5	12–16 April (1 on 1 March)
Red deer (*Cervus elaphus*)	150·0–170·0	4	8–15 Jan.
	190·0–215·0	9	6–20 Jan.
	300·0	1	14 Jan.
	730·0	1	1 June
Père David's deer (*Elaphurus davidianus*)	100·0	1	19 Feb.
	275·0	1	9 March
	330·0	1	10 Jan.
	360·0	1	10 Jan.
	380·0	1	13 Jan.
Manchurian Sika deer (*Capreolus bedfordi*)	241·0	1	10 March
Japanese Sika deer (*Cervus nippon*)	332·0	1	5 April
Indian spotted deer (*Cervus axis*)	420·0	1	12 April
Reindeer (*Rangifer tarandus*)	260–265	4	Not known
	270–280	4	Not known
	294	1	Not known
Roe deer (*Capreolus capreolus*)	12·0–20·0	3	16 Feb.–10 Mar.
	50·0–52·0	2	16 Feb.
	91·0–105·0	4	5–16 Feb.
	110·0–170·0	14	19 Feb.–10 Mar.
	216·0–235·0	3	10 March

The third group of specimens were those pregnant uteri which were fixed unopened in 10 % formalin, or in which the uterus was opened and the foetus or chorionic sac removed for subsequent fixation and preservation (see also details given by Harrison & Hyett, 1954). Sections were also prepared of the ovaries, uterus and vagina of the non-pregnant animals and stained with Heidenhain's haematoxylin and eosin. Samples of the amniotic and allantoic fluids were removed from pregnant uteri at different stages of pregnancy and were analysed for total reducing substances and fructose (Walker, 1954).

REPRODUCTION IN THE CERVIDAE

Observations on the reproductive patterns exhibited by the Cervidae have been made by Lydekker (1898), Heape (1901), and Seton (1909) and the review by Asdell (1946) summarizes much of the earlier information. Lydekker points out that 'many of the larger and more specialised deer are comparatively slow breeders, for although the hinds frequently, if not generally, breed annually, yet it is seldom that more than a single fawn is produced at birth, twins being rare, while triplets are practically unknown'.

The majority of deer are monoestrous and have a limited mating season from September to October. The young are born from April to June. There are, however, a number of exceptions to this generalization. Sambar deer (*Cervus unicolor*) and Mule deer (*Odocoileus hemionus*) do not mate until November or December. Roe deer (*Capreolus capreolus*) mate in July and August and parturition does not occur until the following May. A few species have no fixed breeding season. Indian spotted deer (*Cervus axis*) will breed at any period of the year and in captivity have dioestrous cycles throughout the year. The Red Brocket (*Mazama americana*) and the Muntjacs (*Muntiacus*) also have no fixed breeding season. In Thameng (*Cervus eldi*) the mating season lasts from the middle of March to the middle of May and parturition occurs in October and November.

The following observations give the general pattern of reproduction of the species which we have examined.

Mating in *Dama dama* in parks in southern England occurs throughout October, but may be extended into November. Recently ruptured follicles have been observed by us in December and once in early January. Parturition occurs in the period late May to July. The number of young is one; twins have only been found once in over 300 pregnant uteri examined. The gestation period is 230–240 days; there is no evidence of delayed implantation. Unfortunately we have no information whether the late mating was in animals born late in the previous season or in animals which were suckling their young for a longer period.

Mating in *Cervus elaphus* occurs in September and October, and parturition in May or June with a gestation period of 225–246 days: it is said to exhibit cycles in captivity. A continuous series of cycles of 3 weeks' duration is exhibited by *Cervus axis* in captivity (Heape, 1901) and the late Duke of Bedford stated that breeding occurred at any time of the year at Woburn (Bedford & Marshall, 1942). Gestation lasts 210–238 days.

Mating in *Elaphurus davidianus* occurs in captivity in England in June and July with parturition in mid-April to May; the latest date for parturition is September. *Capreolus bedfordi* is said to mate in July and parturition takes place in May or June. In *Cervus nippon* the gestation period is estimated to be 222–246 days and in *Rangifer tarandus* the period is 216–246 days.

Mating in *Capreolus capreolus* occurs in July and August, there is usually delayed implantation until December or January, but implantation can immediately follow ovulation (Prell, 1938). From the time of nidation pregnancy lasts 140–165 days, and young are usually born in May. Twinning (of like sex) appears to be the rule.

Twinning

Twinning has been found consistently in the European roe deer and once only in fallow deer in the specimens that we have examined. Of the twenty-six pregnant uteri of the roe deer examined, twenty-two of these contained twins; ten of the twin specimens had two corpora lutea in one ovary and ten had one corpus in each ovary, two had only one corpus luteum. All the twins were of similar sex. Lydekker (1898) reports that this species may produce one or two at birth and states that some of the older writers reported that the twins 'are always male and female'. In Manchurian roe there are usually twins.

In the genus *Odocoileus*, twins are generally found in the following species: Virginian roe (*Odocoileus virginianus*), Mule deer (*Odocoileus hemionus*) and Black-tailed deer (*Odocoileus columbianus*). In the Red Brocket (*Mazama americana*) a pair of twins are produced annually. In Himalayan Musks (*Moschus moschiferus*), Sambar deer (*Cervus unicolor*), American Wapiti (*Cervus canadensis typicus*), Indian Muntjac (*Cervus muntjac*) and in the Moose (*Alces alces*) one or two fawns may be produced. The Chinese water deer (*Hydropotes inermis*) is an exception to most deer in that it usually produces three or four young at birth.

PLACENTATION IN FALLOW DEER (*DAMA DAMA*)

Early stages in placentation

The largest number of early specimens available (see Table 1) are from *Dama dama*, but even in the youngest embryo (1 mm. overall length) an amniotic cavity has been formed and the allantois is large. No statement can therefore be made as to the method of formation of the amniotic cavity. The elongated chorionic sac of *Dama* fully occupies both horns of the uterus by the 7 mm. C.R. stage. That part of the sac in which the foetus is lying is much wider and longer, and the uterine horn containing it more swollen than that on the opposite side (Pl. 4, figs. 17, 18). The foetus usually lies nearer the cervix than the mid-point of the pregnant horn. The chorionic sac is transparent and elastic and tapers towards its extremities. The large allantois distends the chorion and is intimately apposed to it except where the amnion intervenes. The allantois increases steadily in size during gestation and from about the 20 mm. C.R. stage it ruptures through the tips of the chorionic sac to form an atrophic region, placed at the tubal extremity of each horn (Pl. 4, fig. 18).

Discrete circumscribed regions, or 'plaques', usually oval in outline, appear on the mesometrial aspect of the chorion from the 12 mm. C.R. stage (Pl. 4, fig. 17). They lie closely related to the elevated maternal caruncles and are the sites of development of the primitive villi of the cotyledons. Small folded elevations of chorion, the areolae, develop on the intercotyledonary regions from the 15 mm. C.R. stage. They are evenly distributed about 1 mm. apart, and appear to lie opposite the mouths of uterine glands. They measure 0·5–0·8 mm. in diameter at the 20 mm. C.R. stage and increase only slightly in size as pregnancy advances. No intercotyledonary villi have been observed.

The umbilical vessels are well developed by the 20 mm. C.R. stage and one artery and one vein extend from each side of the umbilical cord along the lesser curvature or mesometrial aspect of the chorion towards its tips. The umbilical vessels run close to the developing cotyledonary regions and give off branches that pass circumferentially about the chorionic sac. These branches subdivide to supply the cotyledonary areas and continue to supply the intercotyledonary regions. A fine capillary plexus develops in the cotyledonary areas by the 22 mm. C.R. stage. The branches of the main veins run independently of those of the arteries.

Important changes occur in the chorion and the uterus during early pregnancy in all species of Cervidae. Villi develop on the circumscribed areas of the chorion to give rise to the primitive *cotyledons*. The villi are at first short and simple, later they elongate, branch and form tufts of long, filiform processes arising from a single main stem villus. They soon penetrate into *crypts* that develop in the uterine caruncles. These are flattened, sessile, ovoid structures lineally arranged along the mesometrial surface of the inside of the uterine horns. They are devoid of glands. Each cotyledon develops opposite a caruncle and when the villi have become established within the crypts a *placentome* is formed (Pl. 1, fig. 2). As pregnancy advances growth changes occur in the villi, the caruncles and later in the placentomes.

Villi appear first at about the 12 mm. C.R. stage and are arranged in a characteristic pattern. They arise as projections from small ridges, the majority of which lie transverse to the long axis of the cotyledon (Harrison & Hyett, 1954). These authors give details of the origin, number and arrangement of villi on a cotyledon. The growth and relationships of the villi and crypts are described later. No haematomata have been observed in relation to the chorion as Wimsatt (1950) found in sheep and Harrison & D'Silva (1956) in goats.

The structure of the chorion

The chorionic epithelium is composed of two main types of cell even in the youngest specimen (embryo 1 mm. overall length). They are (*a*) the columnar trophoblastic cells which form the majority and are arranged at first as a continuous sheet, 2–3 cell layers thick, and (*b*) the characteristic binucleate cells (or diplokaryocytes) which are distributed at intervals all over the young avillous chorionic sac and which have been extensively studied in ruminants (Wimsatt, 1951). This general arrangement is maintained throughout pregnancy except in certain regions of the villi (page 11).

The columnar trophoblast cells are 15–20 μ in height and 10–15 μ across: their spherical nuclei are 4–6 μ in diameter. In haematoxylin- and eosin-stained sections their granular cytoplasm is moderately eosinophilic and contains minute vacuoles. Nuclei are lightly stained, have a 'linum reticulum' and two or more nucleoli. Minute lipid droplets can be seen scattered throughout the cytoplasm in frozen sections. Alkaline phosphatase (Na-β-glycerophosphate substrate) is demonstrable as a band on the outer region of these cells. No PAS-positive material is present and no acidophilic proteinous crystals have been seen.

The fully differentiated binucleate cell is spherical, 15–20 μ in diameter, with two or more spherical nuclei up to 10 μ in diameter. They display similar cytological characteristics throughout pregnancy (Pl. 3, fig. 16). Chromatin in the nuclei is arranged in striking clumps and gives a marked Feulgen reaction. No distinct nucleoli have been observed. A 'Golgi zone' comparable to that described by Wimsatt (1951) can be demonstrated close to the nucleus.

The cytoplasm throughout most binucleate cells appears to exhibit even distribution of staining properties, but in a number it can be divided into predominantly basophilic supranuclear and acidophilic infranuclear portions (Wimsatt, 1951). A dome-like protuberance is frequently seen on the supranuclear uterine surface of binucleate cells lying superficially in the chorionic epithelium. This and the supranuclear cytoplasm has a distinct basophilic component, but there is often an acidophilic zone in the protuberance. It must be emphasized that this protuberance is only clearly seen when the trophoblast has separated from the crypt lining, and may well be an artefact. One or more large lipid droplets are frequently seen in the cytoplasm. Alkaline phosphatase (Na-β-glycerophosphate substrate) is distributed evenly throughout the cytoplasm, but is scanty or lacking in the nuclei. Some binucleate cells, however, possess only sparse amounts of the enzyme. No maternal erythrocytes, non-ferruginous pigments, or strongly acidophilic proteinous crystals as described by Wimsatt (1951) have been identified in binucleate cells of any deer placenta we have examined.

The binucleate cells give a strong and characteristic reaction with the PAS procedure (Pl. 4, fig. 21). Repetition of the analysis made by Wimsatt (1951) shows that the reacting material is a polysaccharide-protein complex. There is not the same infranuclear restriction of distribution of the material as in the sheep. It appears to be distributed evenly, as in the cow, throughout the cell except for a clearer area that may well correspond to the Golgi zone. Those cells at the tips of villi are always the most intensely stained.

Our material allows no statement on the time of their first appearance, but it is clear that an immense number of binucleate cells are formed during the duration of pregnancy. They may either reproduce themselves or arise from trophoblastic precursors: but we have found no evidence for the former hypothesis. Cells intermediate in form and characteristic between those of typical trophoblast cells and binucleate cells may be seen everywhere in the early chorion. In general the youngest and healthiest binucleate cells are seen in the inner part of the trophoblast (Wimsatt, 1951; Harrison & Hamilton, 1952). These cells contain only one large nucleus, but their abundant cytoplasm, PAS-positivity and other tinctorial, cytoplasmic and nuclear characteristics allow them to be readily distinguished. Nuclear division occurs mitotically without cytokinesis and two nuclei, more rarely three, are formed. At this stage the cells lie nearer the surface of the trophoblast and their plasma membrane makes contact with the crypt epithelium. The characteristic protuberance may now be seen and it is our contention that this may represent the start of an invasive process by which the binucleate cell enters the crypt epithelium (page 10).

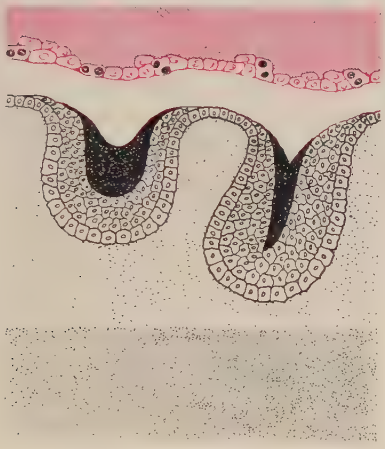
The caruncle

The changes in the caruncle in the earliest stages of pregnancy have only been observed in detail by us in *Dama dama*. A few fairly young stages (12 mm. C.R. length) of *Capreolus capreolus* have been examined and also later stages of *Cervus elaphus* (Table 1). The appearance of these specimens, although at later stages, suggests that the caruncular changes and subsequent placentome formation would not be dissimilar from those in *Dama*. The quite different internal construction of the older placentomes of *Rangifer tarandus* precludes any such assumptions for this species.

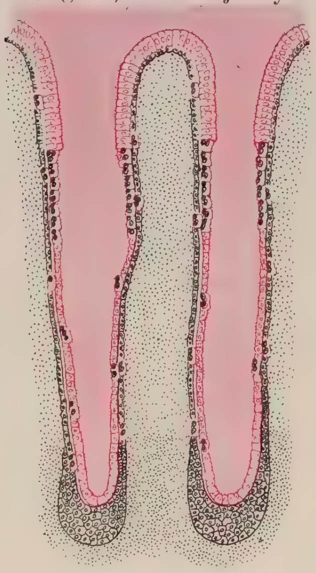
The appearances of the non-pregnant uterus of *Dama* have been described by Harrison & Hyett (1954). The caruncles are arranged in a row along the mesometrial aspect of the uterine lumen. They are elongated, sessile and leaf-like in immature animals; the central ones in each horn are the largest and up to 2 cm. in length and 8 mm. across. They become elongated ovoids supported by a short pedicle in older does. The average number is four in each horn (range 3–6). Each caruncle is covered by a low, single-layered cubical epithelium (Pl. 2, fig. 10). The cells are 6–10 μ in height and have dense eosinophilic cytoplasm with a densely stained nucleus situated centrally in each cell. No evidence of secretion is seen in the caruncular epithelium and none of its cells contains glycogen. The subjacent stromal tissue is devoid of glands and is densely cellular. It does not exhibit any strata of varying density. It is poorly vascularized, but many large, spiral vessels lie in the basal region. The caruncular tissue is not oedematous and contains few leucocytes. The closely packed stromal cells are surrounded by a dense feltwork of reticulin fibrils. Little glycogen or Schiff-positive material is discernible in caruncular stromal cells in the non-pregnant state. In those animals that had been pregnant the previous season the basal region of the caruncle exhibits the fibrous remnants of large obliterated blood vessels that were presumably those supplying the maternal septa.

Placentome formation does not commence until the embryo has surpassed a C.R. length of some 2 to 3 mm. Even at 7 mm. only the central caruncles of each horn exhibit any evidence of crypt formation. Changes occur first in the central region of each caruncle. The caruncular epithelium becomes thicker, the cells enlarge to some 20 μ in height, become two-layered in places, and their nuclei become enlarged and vesicular. Rows of solid epithelial ingrowths develop from the caruncular epithelium. They are arranged lineally, at intervals of about 1 mm. transverse to the long axis of the caruncle (Harrison & Hyett, 1954). The ingrowths are at first conical or spherical, only some 100 μ deep and 50 μ in diameter, and are composed of hypertrophied epithelial cells (Pl. 2, figs, 7, 8; Pl. 4, figs. 18, 20). The ingrowths increase in size until some 0·2 mm. in greatest diameter, when the central cells exhibit degenerative changes. Their nuclei are fragmented and their cytoplasm is dense and aggregated. The central zone is occupied by strongly Schiff-positive material which is partly glycogen but mostly of a glycoproteinous nature. The central region next obtains communication with the uterine lumen and the Schiff-positive material is lost, either by absorption *in situ* or by drainage to the exterior, where it is possibly absorbed by the trophoblast. The latter lies, at this stage, in loose contact with the surface of the caruncle and is presumably held against it by pressure exerted by the ever-increasing size of the allantoic sac. We can find no evidence that it is the development of the chorionic villi that initiates crypt formation. Distinct villi appear to develop only after crypt formation has advanced to some extent. There is no doubt, however, that folds occur in the chorion very easily after death, and it is impossible to be sure that small folds or protuberances of the fixed chorion do not represent primitive villi, although they do not look like them.

Each crypt becomes elongated as it grows, but remains between 0·1 and 0·25 mm. in diameter throughout its length. Those crypts in the centre of the central caruncle in each horn enlarge first. Even by the 20 mm. stage the smaller caruncles at each end of a horn only show early stages of crypt formation. The primitive crypts are invaded by definitive villi as soon as the central cellular debris has started to disappear. The tips of the villi extend into the remnants of this material at the bases of some crypts, even where they have reached a depth of 0·6 mm. The crypts are at first lined by a single layer of cubical epithelial cells, 12–15 μ in height, and with distinct cell boundaries. The layer is continuous with the covering of the surface of the caruncle. The lining at the bases of the crypts is often several cells thick; this may indicate further growth in depth (Pl. 2, fig. 9; Pl. 4, fig. 22). The majority of these cells appear healthy, with a vacuolated cytoplasm and with vesicular nuclei 6–10 μ in diameter. Others have dense, pyknotic, shrunken nuclei. Interspersed amidst these cells are some binucleate cells which can be recognized by their larger size (15–20 μ in diameter), less heavily stained cytoplasm, dense nuclei and polyhedral or oval shape. They also contain Schiff-positive material whereas the lining epithelium has little or none. Alkaline phosphatase activity can be demonstrated in the cytoplasm and these cells are in every way identical to the binucleate cells of the trophoblast (page 7): the significance of their presence in the crypt lining is discussed on page 29.



Text-fig. 1



Text-fig. 2

Text-fig. 1. Diagram to show the method of formation of the primitive crypts in *Dama*. Binucleate cells are present in the trophoblast, but not in the epithelium of the caruncle which is intact and shows downward invaginations with necrotic centres.

Text-fig. 2. Diagram to show primitive villi within developing crypts in *Dama*. Note the hyperplasia at the base of each crypt.

The stromal tissue surrounding the crypts shows little change during early crypt formation. When the crypt has reached a depth of 0·5 mm. there is some evidence of increase in density of that tissue about the tip of the crypt. Intercellular material is faintly Schiff-positive, and there is a slight decidual reaction. The caruncle now begins to exhibit three zones. A superficial zone, nearest the surface epithelium, is 1–2 mm. thick and contains the deepening crypts. It is oedematous and capillaries lie in it parallel to the long axis of the crypt and extend towards the surface. A narrow intermediate zone of young, cellular stromal tissue is only 0·25 mm. thick. Its appearance and the presence of occasional mitoses suggest that it is a zone of active growth. It is perforated by small arteries and veins that branch into capillaries of the superficial zone. A basal zone, varying in thickness from 1 to 5 mm., blends with the loose connective tissue lining the muscle of the uterine horn. It contains fibroblasts, collagenous fibres and many large spiral vessels. The general arrangement of the developing placentome is shown in Text-figs. 1–4 and illustrated in Plates 2 and 4.

Later changes in the crypts

The crypts are up to 2 mm. in depth and 0·25–0·5 mm. in diameter in the largest caruncles by the 27 mm. C.R. stage. They are then lined by cuboidal cells two to three layers thick on the sides of the crypt; there is a denser accumulation of cells at the bases. Binucleate cells invade the crypt lining in considerable numbers at this stage. There is at first no evidence of lining destruction; the binucleate cells appear to probe the lining by insinuation of their cytoplasm between the cuboidal lining cells. Eventually they become intercalated in the lining where many can be distinguished by their intense PAS-positivity; they are supported by a strongly PAS-positive basement membrane. Capillary loops are now arranged close to this membrane. The stromal cells of the caruncular tissue surrounding the crypts are enlarged and vacuolated. Tissue oedema is present and droplets of glycogen are scattered throughout the superficial areas of the caruncle.

Many binucleate cells present in the crypt lining have little or no PAS-positive material. Others show varying degrees of nuclear degeneration, some possess more than two nuclei; occasional mitotic figures can be observed in these cells. Some cuboidal cells also show evidence of nuclear degeneration (pyknosis) and some are collapsed or distorted in shape. Areas of extracellular eosinophilic amorphous material (PAS-positive, but not removed by diastase) are present at the junction of the trophoblast and the cuboidal cells.

From the 30 mm. to the 70 mm. C.R. stage the crypt lining appears to contain: (*a*) cuboidal cells that are considered to be derived from those maternal cells that originally lined the primitive crypt; (*b*) cuboidal cells undergoing degeneration; (*c*) invading binucleate cells, possessing abundant glycoprotein, that are foetal in origin; (*d*) binucleate cells, with little or no glycoprotein; some are degenerating and are intercalated amongst the cuboidal cells. No leucocytes are present and there is no evidence of haemorrhage from maternal vessels.

The fully differentiated villus

The final form and characteristics of a fully differentiated villus in *Dama dama* are shown in Text-fig. 4. The overall shape is filiform and a villus measures up to 25 mm. in total length. The main stem of the villus is 5–6 mm. in length and 1–2 mm. in diameter, and divides into two to six secondary villi, 10–15 mm. in length. These taper gradually from 0·4–0·5 mm. at their origin to 0·2–0·3 mm. at their point of division into short tertiary villi 3–5 mm. in length. Three zones, each with special trophoblastic characteristics, are recognizable and it is our conviction that they each have different functions. They are: (*a*) a storage zone; (*b*) a zone of attrition; (*c*) a zone of physiological exchange (Text-fig. 4).

The storage zone

This is represented by the trophoblast of the great part of the main-stem villi and the arcades between them (Pl. 3, fig. 11). The trophoblast forms a single layer of tall, narrow columnar cells up to 60 μ in height. Their nuclei lie at the base or in the central third of the cells. The apices of these cells are frequently distorted by protuberances or knobs of cytoplasm projecting from the surface (Pl. 3, fig. 14). There is a PAS-positive basement membrane, deep to which is a row of mesenchymal cells; only a few capillaries are present. Binucleate cells are absent in this zone. Numerous droplets of PAS-positive material are present within the apical cytoplasm and in the cytoplasmic protuberances; many of the droplets lie outside the cells (Pl. 3, fig. 12). Diastase treatment and other tests indicate that the greater part of this material is glycogen. The amount of glycogen present increases as pregnancy advances. This zone of the villus may or may not be in contact with the necrotic tissue of the maternal crypt (page 14).

There is an abundant distribution of alkaline phosphatase (Na-β-glycerophosphate substrate) as a band along the outer surface of the columnar trophoblast. It is particularly marked in the distal part of this zone and here there is some intracellular distribution within individual cells. The surface band is less marked, or absent, in the arcades between villi, but there is a narrow distribution along contiguous cell membranes. In places degenerated debris of giant cells may be seen lying against the edges of the trophoblast.

The zone of attrition

This is a narrow zone extending for only 0·5–1·0 mm.; it frequently includes the point of division of the main stem villus and extends a short way down the secondary villi. It is the region of the definitive villus that obtains intimate contact with the crypt wall. The single layer of tall columnar cells with abundant glycoprotein content is abruptly replaced by a double- or treble-layered trophoblast (Pl. 3, fig. 15; Text-figs. 3, 4). This consists of columnar trophoblast cells with many binucleate cells amongst them. The former contain no PAS-positive material, the latter have fine PAS-positive granules distributed throughout their cytoplasm. A fine PAS-positive basement membrane supports the trophoblast and in places it sends slender folds between its cells. The trophoblast separates easily from the crypt lining but where it has not separated it appears to be in contact by processes of varying thickness between which lies granular eosinophilic material (page 14).

The zone of physiological exchange

This zone comprises the remainder of the villus and it is throughout in contact with the crypt lining. The exact nature of this contact is obscure, but under the light microscope it appears to be an edge-to-edge contact by cytoplasmic processes from the trophoblast cells separated by vacuoles and small lakes of amorphous eosinophilic material (page 17). When the trophoblast separates from the crypt lining, as it does only too readily after death, its outer margin has an irregular appearance (Harrison & Hamilton, 1952, pl. 4, fig. 13). The trophoblast has characteristics similar to the zone of attrition, except that (*a*) the binucleate cells are fewer and progressively contain more PAS-positive material towards the tip of the villus; (*b*) intra-epithelial capillaries are present and (*c*) there is an interlocking of microvilli (page 15).

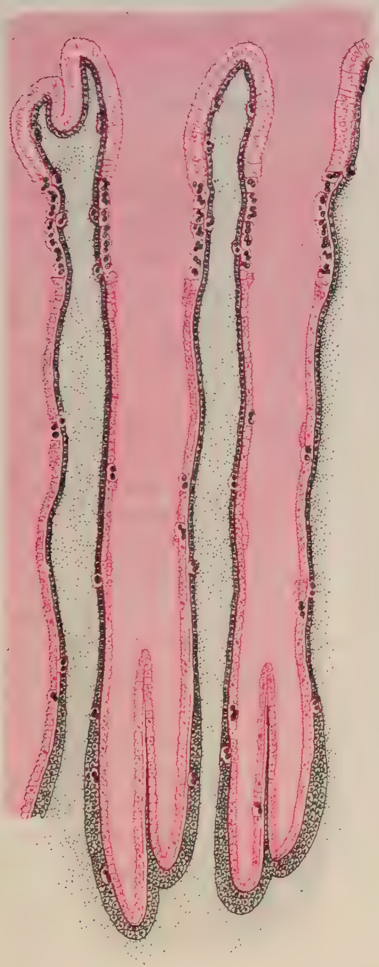
Throughout the zone binucleate cells may be seen deep in the trophoblast, at its surface and in an intermediate position (Pl. 3, fig. 16). Some of them can also be seen invading the crypt lining and lying deep in it in contact with the maternal connective tissue. Many of these cells appear healthy, but many show degenerative changes in one or both nuclei. Alkaline phosphatase (Na-β-glycerophosphate substrate) is distributed in a broad band along the region of contact between the trophoblast and the crypt lining (Pl. 3, fig. 13). The edge of the trophoblast appears to possess the greatest quantity, but there is a marked intracellular distribution in many but not all of the binucleate cells. This continuous phosphatase barrier is not found in the placentomes of the sheep and cow (Wimsatt, 1951).

The crypt lining in relation to the differentiated villus

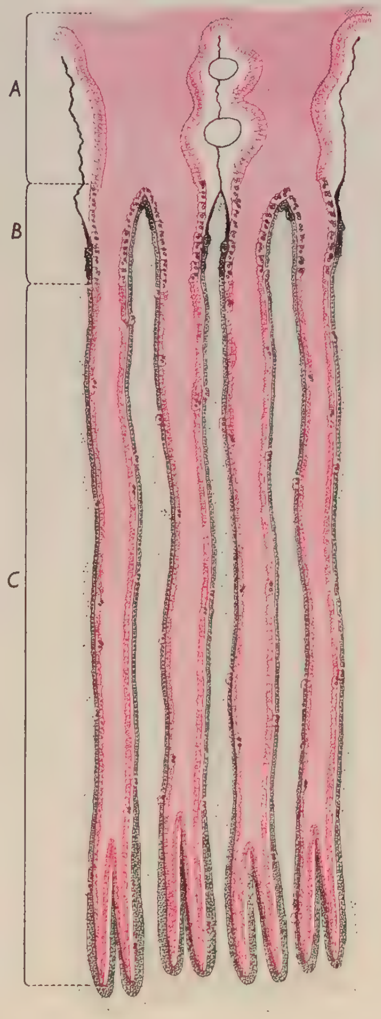
The crypt lining starts to become differentiated into three zones at the 70 mm. c.r. stage. These zones correspond spatially to those already described for the villus (page 11); they are clearly present by the 90 mm. c.r. stage and persist until term. The deepest part of each crypt is subdivided into short basal recesses that receive the tertiary villi (Pl. 3, fig. 16). These regions of the crypt, and the much longer region above it which encloses each secondary villus, maintain the same characteristics throughout the rest of pregnancy. The crypt lining is from one to two cell layers thick except at the bottom of the subsidiary crypts, where it is often thicker. In any specimen several types of cell may be seen in the crypt lining. In younger specimens the lower cuboidal type predominates, but as pregnancy advances more and more of the crypt lining becomes syncytial in character. Numbers of degenerating cuboidal cells can be seen in the fundi of the crypts, particularly in stages from 100 to 500 mm. c.r. length. Some of these cells retain their cuboidal shape and exhibit nuclear degeneration; others are distorted and both cytoplasm and nucleus are fusiform. Mitotic figures are present in some binucleate cells and may occasionally be seen in the cuboidal cells. The binucleate cells contain abundant PAS-positive glycoprotein and cytoplasmic alkaline phosphatase before they enter the crypt lining. Some of those binucleate cells incorporated in the lining retain these characteristics, but the majority possess only small amounts or no glycoprotein. Alkaline phosphatase is found only at the junction between the lining binucleate cell and the trophoblast.

The tissue of the septa consists of a PAS-positive basement membrane supporting the crypt lining, stromal cells and a few fibroblasts. Little collagen is present; a reticular network enmeshes the stromal cells. A network of capillaries surrounds each crypt and lies immediately subjacent to the basement membrane. The endothelial cells are thin and contain alkaline phosphatase; a PAS-positive membrane surrounds the endothelial cells (page 17).

The lining of the crypts changes markedly in its characteristics in the regions opposite the 'attritional' intermediate zone of the villus (page 11). This region of the crypt really represents its true mouth, as the maternal tissue beyond it exhibits degenerative changes. It is a restricted zone and extends only for 1 mm. at the most.



Text-fig. 3



Text-fig. 4

Text-fig. 3. Diagram to show the branching of villi and development of crypts in the early placentome of *Dama dama*.

Text-fig. 4. Diagram to show the branching of villi and destruction of maternal septa in a definitive placentome of *Dama dama*. Three zones are shown in the villus: (*A*) zone of storage, (*B*) zone of attrition, (*C*) zone of physiological exchange.

All evidence of cuboidal cells is lost in this zone and the only cells observable are giant cells of various types (Pl. 3, fig. 15). Healthy binucleate cells with high PAS-positivity are present in the deeper parts of the zone. There are also many areas occupied by large masses of syncytium or plasmodial masses, the characteristics of which differ markedly from syncytial areas deeper in the crypt. These masses are often up to 80 μ in length and 40 μ across and may contain as many as 30 nuclei in one section. They are usually separated from each other by distinct boundaries or indentations, but in places two or more masses appear to be continuous through slender bridges of cytoplasm. The cytoplasm is conspicuously eosinophilic and densely granular; it does not contain PAS-positive material. Alkaline phosphatase in abundant quantities is distributed throughout the cytoplasm and is most concentrated at the maternal border. The nuclei are characterized by their dense basophilia, and not infrequently more than one shows pyknotic changes. Mitotic figures have not been observed despite careful searching of many sections.

Binucleate cells of characteristic appearance are abundantly present in the trophoblast and in the region between the trophoblast and syncytial masses. The syncytial masses nearer the surface of the placentome frequently exhibit degenerative changes. Karyorrhexis and pyknosis occur and the whole mass often breaks away from the crypt wall. The crypt wall becomes altogether denuded of syncytium, binucleate and cuboidal cells. Characteristic changes are present in the tissue of the maternal septa in this region: the endothelial cells of the capillaries are shrunken, the nuclei slender and pyknotic; and no blood is present in the much compressed lumen. Stromal cells and fibroblasts have pyknotic nuclei and, higher in the septa, lose their nuclei completely.

The superficial parts of the maternal septa exhibit striking changes, particularly at the tips (Pl. 5, fig. 24). The septa are devoid of an epithelial covering and show varying degrees of cellular degeneration. The tips of the septa are necrotic, nuclei are absent and the tissue consists of amorphous, eosinophilic material with some reticular and collagenous fibres embedded in it. The material displays less and less PAS-positivity towards the tip; some glycogen is present as droplets lying against the wall. Alkaline phosphatase is abundant in the deeper parts of the septa but is altogether absent in the necrotic tip region.

The terminal parts of the maternal septa at the 78 mm. C.R. stage are still covered by epithelium and show no evidence of degeneration. They are devoid of epithelium for a terminal length of 3–4 mm. and have necrotic tips extending for 1·5 mm. at the 100 mm. C.R. stage. At the 175 mm. C.R. stage the area devoid of epithelium is 4 mm. in length and the necrotic region is 2 mm. in length and is reduced to a fine filamentous thread. Near term, at the 290 mm. C.R. stage, the terminal region has increased to 5 mm. in length and the greater part of it is necrotic and finely filamentous.

This terminal region, particularly the necrotic part, fits loosely between the bases of the main-stem villi and it is frequently plicated. If the chorion and as much of the villi as possible be stripped from a fresh placentome and the exposed surface of the caruncle be placed under water the necrotic filamentous, terminal portions wave freely about the more solid mass of the caruncle.

Details of placentomes in a series of Dama

An analysis was made of the characteristics of the placentomes in the uterine horns of a series of 81 fallow deer pregnant with foetuses varying in size from 85 to 350 mm. The corpus luteum was in the ovary on the same side as the uterine horn containing the foetus in 72 % of the specimens; transmigration of the ovum had therefore occurred in 28 %. Migration occurred from the left to the right horn four times as often as from right to left. The commonest number of placentomes was eight and the commonest arrangement was four in each horn. The greatest number was fifteen (nine in one horn) and in two does the least was four, but the horn without the foetus contained no placentomes. There was no significant difference between the number of placentomes in either horn whether one horn contained the foetus or not. The largest placentomes were found in the pregnant horn in 54 % and the total gross volume of the placentomes was greater in the pregnant horn in 60 % of the specimens.

There was no increase in the average number of placentomes as pregnancy advanced. There were small accessory placentomes only a few millimetres in size in a few specimens both early and late in pregnancy and there was no evidence of neo-formation of small placentomes during pregnancy. The general tendency is for the larger placentomes to become nearly equal in size towards the end of pregnancy (Pl. 1, fig. 3). Placentomes in specimens near term may vary in length and breadth by several centimetres, but they do not exceed 4·5 cm. in height.

Fine structure of the trophoblast and crypt lining in Dama

The structure of the trophoblast, of the cells lining the crypts and the junctional region between the two at the 200 mm. C.R. stage have been examined with the electron microscope. The trophoblast, in every region it has been examined, possesses microvilli on its external surface (Pl. 6, figs. 27, 28). In the depths of the crypts they intimately interlock with microvilli on the surface of the cells lining the crypts. Those on the columnar cells of the main-stem villus lie free in what appears to be cellular debris. The microvilli of the trophoblast cells measure 1·4–3·5 μ in length and 1400–2100 Å in width. They occasionally branch and have tapering tips. The microvilli of the cryptal cells have similar characteristics but are slightly smaller (Pl. 8, fig. 39).

The trophoblast in the zone of physiological exchange contains cubical cells with simple cell membranes and giant cells. The apical region of the *cubical cells* between the microvilli and the densely packed mitochondria is about 2 μ thick and contains numerous profiles of what appear to be vesicles often arranged in rows directed towards the microvilli (Pl. 6, fig. 28; Pl. 7, fig. 29). Some vesicles have a dense lining 350 Å thick. The majority of the mitochondria are arranged in a row on the outer aspect of the nucleus (Pl. 6, fig. 28). They tend to be elongated, 2000 Å in width and 20000 Å in length, and are densely osmiophilic. A few mitochondria are sparsely distributed in the perinuclear region. Endoplasmic reticulum is scanty and few vesicles are present other than in the superficial zone. The trophoblast possesses a basement membrane about 2000 Å thick. Foetal capillaries abut against the bases of the cubical cells and also lie within the trophoblast; a cubical cell may almost completely encircle a part of the capillary. The foetal capillaries have a basement membrane about 350 Å thick which is separated from that of the trophoblast cells by a clear space 700 Å thick. The intervening space does not appear to be markedly lamellated as described by Björkman and Bloom (1957) in the bovine placenta. No deficiencies or pores have been seen in the foetal endothelium; small processes or protuberances project into the lumen (Pl. 8, figs. 36, 37).

Binucleate cells have also been recognized lying within the maternal crypt lining (Pl. 8. fig. 30). They possess a few short microvilli (300–2800 Å in length) on the aspect in contact with the villus (Pl. 8. fig. 31). Short clefts are occasionally present between them and neighbouring crypt cells. There is an inclusion-free zone in the cytoplasm nearest the villus. It contains only a few small vesicles. Most of the mitochondria lie in a row between the nuclei and the cell surface nearest the villus. The mitochondria are small, spherical and frequently contain one or more small vesicles. The endoplasmic reticulum is perinuclear and is well developed in some binucleate cells: a Golgi apparatus is present. Vacuoles from 0·3 to 1·5 μ in diameter are present in the inner cytoplasm. They have clear centres. but occasionally a peripheral zone of finely granular material is present. Some binucleate cells have collections of osmiophilic granules which are related to the endoplasmic reticulum. The nuclei show localized aggregations of electron-dense material and also nucleolar-like structures with a reticular pattern.

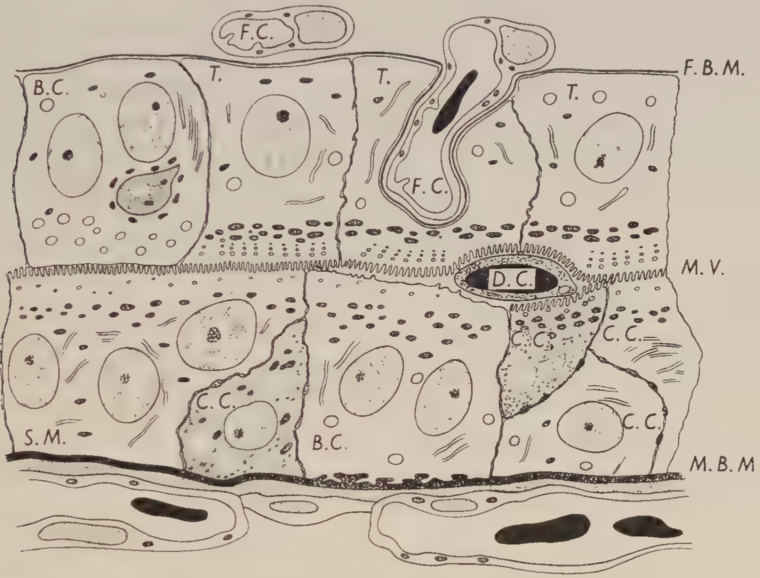
The *columnar* trophoblast cells of the main stem of the villi have a much clearer cytoplasm than that of other trophoblast cells. The adjoining plasma membranes of contiguous cells are simple. but separate near the cell surfaces to provide an intercellular cleft containing microvilli. Mitochondria tend to be aggregated in the superficial regions of the cell; they resemble those of the cubical cells. Small vesicles 350–14000 Å in diameter are present in some number in the superficial zone and more sparsely throughout the rest of the cell (Pl. 8. fig. 38). Endoplasmic reticulum is scanty and little detail is discernible in the cytoplasm apart from sparsely distributed osmiophilic granules.

The crypt lining shows much complexity of structure. is often several cells thick and contains various cell types. In general it appears more electron-dense than the trophoblast. There are simple cubical cells of varying electron-density and exhibiting different types of vacuolation. Binucleate cells are present (page 12) and also regions occupied by a limited syncytium which may or may not be accompanied by other types of cell (Pl. 6, fig. 28).

The cubical cells have a distinct plasma membrane and microvilli on their foetal aspect (page 15). Their cytoplasm may be relatively clear or may be markedly electron-dense. A few small vesicles are present in a narrow superficial zone (up to 1·0 μ thick) below the microvilli. but they are not as plentiful as on the foetal side. The majority of the mitochondria are arranged in a thick row below the inclusion-free superficial zone. The mitochondria appear less dense and somewhat smaller than those in the trophoblast. Endoplasmic reticulum is well developed in some cubical cells (Pl. 6. fig. 27). Many cubical cells contain heavily vacuolated areas; the clear-centred vacuoles appear to be included in the plasma membranes of two contiguous cells. Other cells have plicated plasma membranes where they are in contact.

The appearances of the binucleate cells are described above: those seen in the crypt lining cannot be differentiated from those in the trophoblast. The syncytial regions possess surface microvilli of moderate length (up to 1·0 μ). The clear superficial zone is not marked and contains few vesicles. Mitochondria are small and are arranged in a broad row near the foetal surface, but some are present throughout the cytoplasm. Endoplasmic reticulum is present in all regions of the syncytial masses. Vacuoles are small and few in number.

Maternal capillaries lie deep to the crypt lining and are separated from it by a space up to 5000 Å thick, and a basement membrane to the crypt lining about 1800 Å thick. Small projections from the endothelial cell may enter the intervening space, which contains irregular heterogeneous material (Pl. 8, figs. 34, 35). In places binucleate cells extend as far as a maternal capillary and the plasma membrane of the binucleate cell may show small infoldings along this margin.



Text-fig. 5. Diagram to show the structure of the trophoblast (above) and the crypt lining in *Dama* as seen with the electron microscope. *B.C.* = binucleate cells, one of which is in the crypt lining. *C.C.* = cuboidal crypt cells of varying electron density. *D.C.* = degenerating cell in junctional zone. *F.B.M.* = basement membrane to trophoblast *T. F.C.* = foetal capillaries, one intra-epithelial. *M.B.M.* = basement membrane of crypt lining with maternal capillaries below it. *M.V.* = microvilli of columnar trophoblast interdigitating with those of the crypt lining cells, but absent from the binucleate cells. *S.M.* = syncytium in the crypt lining.

At intervals the microvillous junction between the trophoblast and crypt lining contains what appear to be degenerating cells. They are markedly electron-dense, with granular and often heavily vacuolated cytoplasm. Their nuclei are particularly dense. Where such degenerating cells are present the adjacent trophoblast is devoid of the characteristic rows of small vesicles (Pl. 6, fig. 28). Remnants, or portions, of such cells may also be seen enclosed by, or included within, the trophoblast (Pl. 6, fig. 28) and the binucleate cells. The relationships of the cell types seen in the trophoblast and crypt lining are illustrated diagrammatically in Text-fig. 5.

PLACENTATION IN OTHER DEER

Japanese Sika deer (Cervus nippon)

A single pregnant uterus of a Japanese Sika deer (*Cervus nippon*) was obtained on 5 April 1951. The almost spherical uterus was 35 cm. in length, 27·5 cm. in breadth and 108 cm. in circumference. The right uterine horn contained a male foetus 332 mm. in C.R. length. Six large placentomes were present, three in each horn. They were approximately spheroidal (Pl. 1, fig. 4) and were mounted on a short pedicle of maternal tissue. Their greatest diameters were:

Horn with foetus (cm.)	Other horn (cm.)
7·0, 6·0, 5·0	9·0, 7·0, 5·0
8·5, 7·0, 6·0	7·0, 5·0, 5·0
5·0, 3·5, 4·0	7·0, 7·0, 6·0

The secondary villi arise from a single squat stem 3–5 mm. in length in the large placentomes. The filiform secondary villi are up to 3 mm. in length and 5 mm. in diameter. Some three to five arise from each main stem villus. They bifurcate into tertiary villi 0·5–3·0 mm. long. The characteristics of the trophoblast covering are essentially similar to those of *Dama* at an equivalent stage of development. The most marked differences are seen in the covering of the main-stem villi and the arcades between them. The trophoblast is tall columnar as in *Dama*, but there is less glycogen in the cells or lying outside them. There is also a markedly increased number of degenerating masses, with many pyknotic nuclei, lying against the surface of the trophoblast.

The lining of the maternal crypts resembles that of *Dama*. The septa can be divided into three zones similar to those described on page 12. The deeper parts of the crypts are lined with varying proportions of simple cuboidal cells with distinct cell boundaries and moderately densely stained nuclei, binucleate cells, and elongated stretches of what appears to be syncytium with densely stained nuclei. The septa lose their epithelial covering in a manner comparable to that in *Dama*. The region of the mouth of each crypt exhibits dense syncytial masses and binucleate cells which send projections of cytoplasm into the septum. These projections appear to penetrate the crypt wall and extend between the stromal cells. All the cells in the septa show degenerative changes in this region. There are necrotic filamentous tips to the septa.

Indian spotted deer (Cervus axis)

A single specimen of pregnant uterus of the Indian spotted deer (*Cervus axis*), obtained on 12 April 1951, contained a female foetus 420 mm. in C.R. length. There were four large placentomes in the pregnant right horn and two large placentomes and one small in the other. The largest placentome was an elongated ovoid, the others were flattened spheres. Their dimensions were:

Horn with foetus (cm.)	Other horn (cm.)
7·0, 6·0, 4·0	6·0, 5·0, 4·0
4·0, 4·5, 3·5	9·0, 5·0, 3·0
5·0, 5·0, 3·0	2·5, 2·5, 2·5
6·0, 4·0, 3·5	

The villi have a general arrangement similar to that of *Dama* in that several filiform villi arise from a stout main-stem villus. The main-stem villi in a large placentome are 1·5–2 mm. long and 0·6–0·9 mm. in diameter. The secondary villi are 8–10 mm. in length and divide into two to four fine tertiary villi 1·5–2 mm. in length. Three zones are discernible in the crypts and villi similar to those described for *Dama* (page 11). The crypts are lined by a single layer of low epithelial cells 8–10 μ

in height which does not appear to be a syncytium. Only occasional binucleate cells are present in the lining of the deeper parts of the crypts. The lining cells at the crypt's mouth show evidence of pyknosis and degeneration and are not present in the most superficial part of the crypt wall. This part shows some evidence of attrition, but it is not marked as in equivalent stages of *Dama*.

The structure and histological characteristics of the villi are generally similar to those in *Dama*. The main-stem villi and arcades between them are lined by cells that are not as large or as tall as the typical columnar cells in this region in *Dama*. There is only a small amount of glycogen in or outside these cells.

There is a compact block of tissue several centimetres in extent in one region of one placentome. The compact arrangement appears to be due to the absence of any mucoid connective tissue in the villi. These are slender, often only 30–40 μ in diameter, and are encased in thick-walled crypts.

Manchurian Sika deer (Capreolus bedfordi)

A single pregnant uterus of the Manchurian Sika deer (*Capreolus bedfordi*) was obtained on 10 March 1951. The uterus was spherical and about 73 cm. in circumference. The right horn contained a foetus 241 mm. in C.R. length. Six large placentomes of approximately similar size were present. They were ellipsoidal in shape, but were markedly flattened against the uterine wall. Each was attached by a short pedicle. Their greatest diameters were:

Horn with foetus (cm.)	Other horn (cm.)
8·0, 6·5, 2·5	6·0, 5·5, 2·0
6·0, 5·0, 2·0	6·5, 5·0, 2·0
5·5, 4·0, 2·0	5·0, 4·5, 2·0

The structure of the placentome is generally similar to that of *Dama* in the late stages of pregnancy. Each placentome is, however, more flattened and the villi are shorter. The plump main-stem villi are 4–5 mm. in length and 0·25–0·5 mm. in diameter. Several (2–4) filiform secondary villi, 10–12 mm. long and tapering from 0·4 to 0·15 mm. in diameter, arise from them. Some secondary villi bifurcate into short tertiary villi 1–1·5 mm. long and 0·15–0·10 mm. in diameter.

The basic construction of the villi resembles that in *Dama* and three zones are clearly discernible in both villi and crypt lining. The main-stem villi and arcades between them possess a covering of high columnar trophoblast rich in glycogen. The superficial region of the crypt wall is necrotic for 3–3·5 mm. and is devoid of a lining. The trophoblast contains many binucleate cells and is several layers thick at the true mouth of the crypt. The crypt lining shows extensive degenerative changes in this region, many degenerating giant cells being included. The remainder of the crypt lining is somewhat thicker than in *Dama*. It is up to 30 μ in thickness in some areas and consists of layers of discrete cuboidal cells 9–10 μ in height with eosinophilic cytoplasm. Binucleate cells are rarely seen in the crypt lining, and when present appear degenerate.

Red deer (Cervus elaphus)

Fifteen specimens of pregnant uteri of the red deer have been examined. All contained single foetuses which varied in c.r. length (Table 1) from 150 to 730 mm. (near term). The number of placentomes varied from six to ten; the commonest number was eight, four in each horn. There were only two placentomes in one horn, four or more in the other, in two animals. Where there were only two or three placentomes in one horn, they were consistently larger than those in the other horn. The placentomes in the other specimens were of almost equal size and were elongated ovoids raised on a short pedicle (Pl. 1, fig. 1). At the 150 mm. c.r. stage they measured about 40 × 35 × 30 mm. in overall dimensions; the largest placentome was not always in the horn containing the foetus. Near term the overall dimensions of the largest placentome were 110 × 70 × 55 mm.

The arrangement of villi within a placentome and the form of the villi exactly resembles that in *Dama*, as do the details of the cellular construction of the trophoblast and the crypt lining. Our findings confirm those of Strahl (1906).

Reindeer (Rangifer tarandus)

Only nine uteri from pregnant reindeer were in a reasonably preserved condition. The foetuses were all singletons, varying in c.r. length from 260 to 294 mm. The placentomes were ovoid or ellipsoid and mounted on short pedicles; a few displayed a kidney-like shape. The usual number of placentomes was six, three in each horn. The greatest number in one horn was five, the smallest three; where the number was greater than three the additional placentomes were much smaller. The corpus luteum was present in the ovary on the side of the uterine horn that contained the foetus. The dimensions of the placentomes in the uterus of a reindeer pregnant with a foetus 265 mm. in c.r. length were:

Horn with foetus (cm.)	Other horn (cm.)
3·0, 2·5, 2·5	7·5, 3·5, 3·5
3·25, 2·5, 2·5	6·5, 3·5, 3·0
0·75, 0·7, 0·3	8·5, 3·25, 3·5
3·0, 2·0, 2·5	
2·5, 2·0, 1·5	

If a large placentome is sliced longitudinally it can be seen to be sub-divided by septa of maternal connective tissue into a series of lobules (Pl. 5, fig. 25). These are pyramidal in form with their apices directed towards the depths of the caruncle (see also Turner, 1878 *a*, *b*; Kolster, 1909; and Andresen, 1927). A broad, short, main-stem villus, up to 2 cm. in diameter, enters the base of each lobule. A large number of branches arise from each main-stem villus. Those nearest the surface of the placentome are straight and directed laterally; those deeper in the placentome are more obliquely arranged and branch repeatedly to form a complicated mass of secondary and tertiary villi (Pl. 5, fig. 25). The laterally directed villi possess an obvious mesodermal core (Pl. 5, fig. 26), whereas those deeper in the placentome possess only a sparse amount of mesoderm. The total overall length of one tuft of villi in a lobule of the largest placentomes at the stages described is between 1·5 and 2 cm. The villi fit into maternal crypts and thus the latter are arranged in a more complicated manner than in *Dama*. The deepest divisions of the crypts are, however, arranged nearly vertically to the base of the caruncle.

Unfortunately the preservation of the specimen is adequate only for a brief description of the cellular structure of the trophoblast and crypt lining. The main-stem villi and the arcades between them are covered by a single layer of trophoblast consisting mainly of tall columnar cells. In places, and particularly on the lateral branches, the columnar cells are replaced by a layer of cuboidal or squamous cells two or three cells thick. Where the more complex branching of the villus begins the trophoblast contains numerous binucleate cells and some cuboidal cells. Towards the extremities of the subsidiary branches the number of binucleate cells is scanty and cuboidal cells predominate.

The main maternal septa that divide the placentome into lobules are relatively stout and are up to 1 mm. thick. Complex folded subsidiary septa extend inwards into the lobule. The superficial ones are horizontal and form crypts for the laterally directed villi. Those septa nearest the chorion are necrotic and devoid of any lining; others slightly deeper in the lobule are lined by syncytial masses and individual binucleate cells and do not show degenerative changes. Masses of degenerating cellular debris are present in the more superficial crypts. The lining of the remainder of the crypts is uniform and appears to consist of a single or double layer of cuboidal cells with distinct cell membranes. In places the lining has disintegrated post-mortem into individual cuboidal cells. Binucleate cells are present within the crypt lining.

Roe deer (Capreolus capreolus)

A total of thirty-two adult roe deer were killed at Petworth Park between 5 February and 10 March 1952 and 1953. Twenty-six does were pregnant (Table 1); one specimen, not included in the Table, contained two macerated foetuses, one at the tubal end of each horn. There were no caruncles in the uterus and it was presumed to be abnormal. Twenty-two of the pregnant does contained two foetuses (one in each horn), four contained singletons. Ten of the twin pregnancies had two corpora in one ovary, ten had one corpus in each ovary and two had only a single corpus luteum. Therefore 84 % of this small series displayed twinning and at least 7·7 % showed either monozygotic twinning or ovulation of two ova from one follicle. The fact that all the twins were of like sex strongly suggests that monozygotic twinning does occur, and that only one egg is in fact fertilized. It was noticed that fusion of the chorion in the twin pregnancies always occurred, but the two circulations could not be shown to anastomose after injection of coloured material into the umbilical vessels of one twin. The two amniotic cavities did not communicate, nor could it be shown that the allantoic sacs were in continuity. It would appear that the fertilized ovum separates at the two-cell stage, and that one of the separated blastomeres migrates to the opposite horn. The chorionic sacs become apposed and the chorions fuse at a later date, but probably before the 20 mm. C.R. stage.

The corpora lutea in those does that were carrying twins and had two corpora lutea show little histological difference and could well have ovulated at the same time. There was no evidence of multiple follicle formation in the ovaries that were examined. Further discussion of twinning in deer will be found on page 5.

The placentomes develop from flattened, sessile caruncles that are nearly circular in outline; later they become spheres or ovoids raised on a pedicle. The villi are only a few millimetres long by the 20 mm. c.r. stage, but are already lying in shallow crypts. The crypts are 4–5 mm. deep by the 80 mm. c.r. stage, but the villi separate easily from them. The usual number of placentomes is eight, with four in each horn. The smallest number in one horn was two, the greatest six. The average diameters of the placentomes at the 230 mm. stage were:

Horn with foetus (cm.)	Other horn (cm.)
5·7, 3·7, 3·3	7·2, 4·0, 3·8
5·1, 3·7, 3·6	6·7, 4·2, 3·0
7·2, 5·0, 4·0	3·6, 3·0, 2·5
5·6, 3·9, 3·5	5·6, 4·0, 3·5
	4·2, 3·3, 3·1

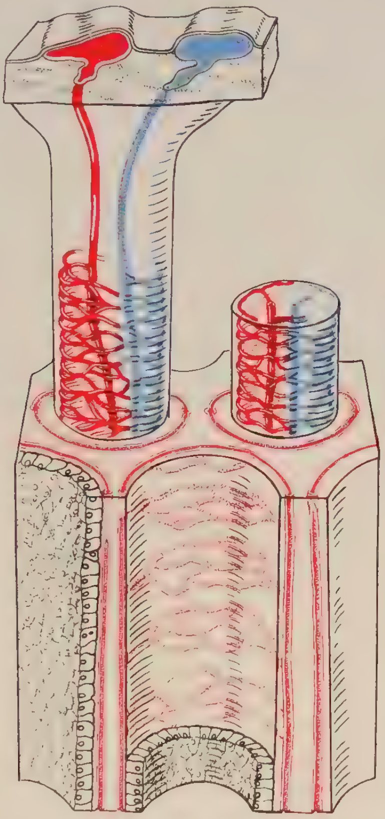
The arrangement of the villi resembles superficially that in *Dama* or *Cervus* rather than that in *Rangifer*. The main-stem villus gives off a number of short branches, however, which all branch again to form tufts of short terminal villi. Some of these terminal branches project laterally into short horizontal crypts, a feature which distinguishes the placentomes of *Capreolus* from those of the Cervinae. The trophoblast displays the same regional differentiation as in *Dama* except that the zone of storage with its tall columnar cells is reduced in extent due to the short main-stem villi. Some columnar cells contain degenerated cellular remnants. The caruncle is subdivided by a few main and many subsidiary septa so that the crypts exhibit a more complex arrangement than in *Dama*. The crypts are lined predominantly by a single layer of cubical cells; occasional binucleate cells are present, and in some regions there is evidence of a syncytium. There does not appear to be the same type of necrotic filament to the tips of the maternal septa as in *Dama* and *Cervus*, although our material does not extend beyond the 235 mm. stage. The apices of the maternal septa may end as rounded knobs (see Andresen, 1927) devoid of epithelium and showing evidence of degeneration (Pl. 5, fig. 23) or as narrow strands still with some cellular organization but with degenerating epithelium. Hyperplasia of the septal epithelium also occurs about the mouths of the crypts but few binucleate cells are present in the crypt lining. Many binucleate cells on the surface of the trophoblast and in contact with these hyperplastic regions have intracytoplasmic inclusions and pyknotic nuclei.

BLOOD SUPPLY OF VILLUS AND CRYPT

The arrangement of the blood vessels in the placentome of *Elaphurus* has been described by Harrison and Hamilton (1952). Similar arrangements were found to exist in the placentomes of *Dama* and *Cervus* which had been injected with coloured gelatin. The structure of the foetal and maternal capillaries as seen under the electron microscope is described on page 15. The following additional observations are emphasized and some are illustrated in Text-fig. 6 and Plate 8, figs. 34–37.

Capillaries are present in the villi of the largest cotyledonary plaques of *Dama* at the 12 mm. c.r. stage and probably earlier. Intra-epithelial capillaries have not been observed until about the 100 mm. c.r. stage. They are present in all Cervidae we have examined and are always covered by narrow strips of trophoblast however closely they extend to the surface.

The foetal endothelium is closely applied to the trophoblast and the intervening space seen in electron micrographs is narrower than that seen between the maternal endothelium and the crypt lining (page 17). The pattern displayed by the capillary network in a villus in *Dama* and *Cervus* is similar to that in *Elaphurus*. There are no intra-epithelial capillaries in the main-stem villi or the arcades between them.



Text-fig. 6. Diagram to show the arrangement of the central artery and vein in a villus of *Dama dama*: note the circumferential anastomosing capillary plexus. The crypt lining has been partially removed from one crypt to show the trellis-like arrangement of the maternal capillaries.

The capillaries are distributed relatively sparsely deep to the columnar cells as opposed to the closely arranged circumferential plexus that is present in the rest of the villus.

Each placentome is supplied by branches of the uterine artery and vein which enter the base of the caruncle. Many, if not all, of the vessels penetrating the basal zone of the caruncle are spiral. Subsidiary arterial branches, which are not spiral, enter the septa vertically and give off short laterally directed arterioles. These divide into leashes of capillaries that extend round a segment of the crypt wall, always deep to the crypt lining and generally in a horizontal plane, i.e. at right angles to the long axis of the villus.

The leash of capillaries join to form a horizontal venule which drains into the vertically placed septal vein (Text-fig. 6). The vertical vessels of the septa are arranged at intervals in the wall of each crypt so that a villus is surrounded by what may be termed a series of trellis-works of maternal vessels.

DISCUSSION

The literature on placentation in ruminants is voluminous (Andresen, 1927; Wimsatt, 1950, 1951; Amoroso, 1952, for references) and no attempt is made here to review or consider all the problems. Certain aspects only are discussed, especially where our material appears to have provided relevant information.

Implantation

The process of implantation, that is to say the mode of attachment of foetal cotyledon to maternal caruncle, has not until now been observed in the Cervidae, although Strahl (1906) examined one specimen of *Cervus elaphus* with an early embryo. In the ewe Assheton (1906) found that the caruncular epithelium was distinctly degenerated in the regions where the chorion made contact and formed a cotyledonary burr. He maintained that the epithelium was not subsequently regenerated but was replaced by binucleate cells. Hammond (1927) reported that in the cow the caruncle was completely denuded of epithelium prior to implantation. Björkman (1954) examined bovine material after fixation or freezing of the chorion *in situ* and found that 'the caruncular epithelium is intact to a large extent during implantation'. We have used similar methods to preserve our material and have found no evidence of denudation of the caruncular epithelium during implantation in *Dama* or of an early invasion or replacement of the superficial caruncular epithelium by any foetal cells.

Björkman (1954) reviews the problem as to how the crypts are formed by downgrowth and subsequent canalization of strands of uterine epithelium, with the villi penetrating them later. In *Dama* the crypts develop before the primitive villi are formed. The caruncular epithelium exhibits localized areas of hyperplasia, the central regions of which become necrotic. The epithelial invagination becomes canalized as a result of disappearance of the necrotic material. The primitive crypts are arranged in rows transverse to the long axis of the caruncle (Strahl, 1906; Turner, 1879; Harrison & Hyett, 1954). The primitive villi enter the crypts after the disappearance of the central necrotic core and only after their establishment within the crypts are any binucleate cells seen within the crypt lining. The subsequent growth changes in the caruncle and villi are described by Harrison & Hyett (1954).

The placentomes

The placentomes in ruminants vary in number, size and shape; there may be as few as five in Père David's deer (oligocotyledonary), or as many as 160–180 in goats (polycotyledonary). The usual number of placentomes in the deer examined by us is six to eight. The largest placentomes have been found in Père David's deer and the smallest in cows. Andresen (1927) has described the different shapes of placentome encountered in ruminants. In deer they all eventually become pedunculated in contrast to the convex, concave or sessile shapes in other ruminants. Andresen considered that the Cervidae have placentomes with a shape specific for each form, but he had not available specimens from stages throughout pregnancy. Our material, in particular that from *Dama* (see also Harrison & Hyett, 1954), shows that the form of the placentome changes markedly as pregnancy advances, and that the shape of neighbouring placentomes in one uterine horn may also vary. There is no correlation between size of placentome and whether its horn contains the foetus as Strahl (1906) suggested. When there are only two placentomes in a horn they are always large.

The construction of the placentomes and the arrangement of the villi in those genera in the subfamily Cervinae (*Dama*, *Cervus*, *Elaphurus*) is remarkably similar. The placentomes of *Rangifer* are differently constructed in that the villi branch in more complex fashion and the arrangement appears to resemble that described for some other members of the subfamily Odocoileinae (*Hydropotes*, Ewart, 1878; *Mazama*, Andresen, 1927). The placentomes of *Capreolus capreolus* display a somewhat intermediate condition (page 21), but as Andresen (1927) observed, may be differentiated from those of *Cervus* by the tufts of short, much-branched villi and numerous lateral branches.

No new placentomes are formed during pregnancy. Small accessory placentomes are formed at the same time as, or shortly after, the main ones, and presumably arise as the result of the existence of small regions of caruncular tissue outside the main caruncles (Kolster, 1903).

We find three distinct zones of cellular differentiation and activity in the definitive villus of *Dama*; they are comparable with similar zones described by Strahl (1906), Kolster (1908) and Andresen (1927) in the placentome. These authors did not, however, possess histochemical and other methods of analysing the functions of the components of each zone. We recognize, from the tip to the base of the villus, (*a*) a zone of physiological exchange (Wachstumzone of other authors); (*b*) a zone of attrition (Umwandlungszone); (*c*) zone of storage (Rückbildungszone). We feel that the division of the placentome into zones is better replaced by an analysis of those exhibited by the villus. We prefer calling the main part of a villus a zone of physiological exchange as that appears to be its main function; growth changes occur everywhere in the placentome as Björkman (1954) also maintains. Electron micrographs show that the trophoblast possesses well-developed microvilli in this zone. Large numbers of small intracytoplasmic vesicles lie in the apical region of the trophoblast cells between the microvilli and the lineally arranged mitochondria. These appearances strongly suggest the presence of a transfer mechanism; enzyme systems are also situated in this region. The zone of attrition is one where the activities of the binucleate cells, and perhaps other factors, cause hyperplasia of the crypt lining, its subsequent destruction and also that of maternal connective tissue with the formation of atrophic filamentous threads on the tips of the septa. The zone of storage is characterized by the tall columnar trophoblast cells of the main stem villi and the arcades between them. These cells have apical microvilli and contain quantities of glycogen and glycoprotein.

The definitive placentome also exhibits three zones in the maternal septa corresponding in position to those analysed in the villus. The greater part of each crypt has a complex lining containing several cell types (see later) most of which take part in physiological exchange. At the mouths of the crypts is a second zone characterized by the presence of large syncytial masses (plasmodial masses of Björkman, 1954) with from three to thirty dense nuclei. Many show evidence of pyknosis and advanced degeneration. This zone lies opposite the zone of attrition of the villus. It has been suggested (Assheton, 1906; Wimsatt, 1950; Amoroso, 1952) that these masses may arise from binucleate cells that have left the trophoblast to enter the crypt lining. Björkman (1954), however, points out that the staining characteristics of the plasmodial masses of the cow resemble those of the cellular crypt lining, and the same is true in *Dama.* We consider these masses to be formed from the crypt lining, and they are probably maternal in origin. The third septal zone is one of complete attrition of maternal tissue. The crypt lining is completely absent and the septal connective tissue is reduced to an atrophic strand. The term diaphthoro-epithelio-chorial was introduced to describe this relationship by Harrison and Hamilton (1952) and has been discussed by Harrison & Hyett (1954) in that it illustrates an adaptation of the placentome in deer for maximal physiological efficiency.

The lining of the crypts

There has been disagreement as to the nature and origin of the crypt lining in ruminants. Andresen (1927) maintained that the lining is often syncytial and that where a syncytium is found it is derived from the trophoblast. This generalization was considered to be premature, and probably erroneous, by Wimsatt (1950). He quotes the opinions of Sedlaczek (1912), Wislocki (1941) and Wislocki & Fawcett (1949) who had examined specimens of the antelopes *Hippotragus bakeri*, *Rhyncotragus kirkii* and *Antilocapra americana.* All had considered that the syncytium lining the crypts was maternal in origin. In the cow Kolster (1902), Jenkinson (1906), Chiodi (1927) and others considered that the lining was derived from modified uterine epithelium. Hammond (1927) maintained the lining was not epithelial in nature but consisted of connective tissue, plasma or lamellar cells. Strahl (1906, 1912) considered the syncytium to be maternal in origin in *Cervus elaphus.* Kolster (1909) disagreed with Strahl and maintained, as did Andresen (1922), that the syncytium also arose from trophoblast in *Cervus elaphus* and in *Rangifer tarandus.* Assheton (1906), on the other hand, considered the syncytium in the crypts of the sheep to be formed from binucleate trophoblastic cells. Kolster (1909, 1910) supported Assheton's contention, as did Andresen (1927) and others. Wimsatt (1949) at first took the view that maternal connective tissue gave origin to the syncytium, but later (1950, 1951), and as the result of careful histochemical investigations, revised his view and supported Assheton's interpretation. Björkman (1954) is convinced that if bovine material is well fixed, distinct cell boundaries may be seen in the crypt lining. He also observed mitoses and states dogmatically that the crypt lining is cellular and of maternal origin. Later he confirmed this statement by using an electron microscope to study the foetal-maternal junction (Björkman & Bloom, 1957).

Harrison & Hamilton (1952) considered the crypt lining in *Elaphurus* to consist of distinct cuboidal cells with definite cell membranes and used phase contrast microscopy to illustrate the lining. The crypt lining varies in the different deer described in this paper from a single row of cuboidal cells to a complex layer containing several cell types. The lining also changes in complexity and construction as the placentome grows. It does not remain static, but is everywhere changing in its composition in that it exhibits growth, suffers invasion and destruction and may even undergo metaplasia.

Electron micrographs confirm our observations with the light microscope that the crypt lining, at least in *Dama*, contains several types of cell. There are numerous *cuboidal cells* which we believe from our study of successive stages in placentome development to be maternal in origin. They undergo mitosis, but also show degenerative changes. They possess microvilli which interdigitate with those of the trophoblast. They are supported at their bases on a continuous basement membrane. *Binucleate cells* are present, and we are convinced that they have migrated there from the trophoblast (see later). Their histochemical characteristics are similar whatever their position, but they appear to lose their PAS-positivity after they have become incorporated in the crypt lining. They also show evidence of nuclear pyknosis and cytoplasmic degeneration when within the crypt lining or in contact with it. They do not possess pronounced microvilli when any part of their surface is included in the region of the foetal–maternal junction. Where their plasma membrane comes in contact with the crypt basement membrane it shows much irregular folding. Certain regions of the crypt lining are definitely syncytial. These regions extend for distances of at least a millimetre and are interspersed amongst cuboidal and binucleate cells. Microvilli are present and both in their staining characteristics and ultrastructural features they resemble the cuboidal cells. For these, admittedly mainly morphological, reasons we consider the limited regions of syncytial lining to be derived from the maternal cuboidal cells. If, on the other hand, they are derived from binucleate cells it means that the latter, after invasion of the crypt lining, lose certain characteristic cytological features and take on those of the cuboidal cells of the lining proper. Neither Björkman & Bloom (1957) nor we have found any evidence for such a transformation.

Binucleate and placental giant cells

The origin, classification and time of appearance of giant cells, Riesenzellen, megalokaryocytes, diplokaryocytes or binucleate cells in the placenta and related endometrium have occupied the attention of investigators for many years. It has been postulated that they may arise from the trophoblast, the decidua, or from the maternal endothelium. Hubrecht (1889), Duval (1895), Sobotta (1903) and Jenkinson (1906) were of the opinion that the giant cells were of trophoblastic origin. Disse (1906) in field mice, and Sansom (1922) in water voles, believed that they were of maternal origin. Sansom (1927) stated that in the rabbit two kinds of giant cells were present, larger cells derived from the trophoblast and mesometrial cells formed from the endothelial lining of maternal vessels.

Orsini (1954), following at least two other investigators (Snell, 1941 and Alden, 1946), has classified giant cells in rodents into three types. Primary giant cells arise by hypertrophy of the abembryonic and lateral areas of the blastocyst wall and play an active role in the penetration of the uterine epithelium. Later in development, the cells become restricted to an area bounded internally by Reichert's membrane and externally by the degenerating decidua; some of the cells may actually penetrate the decidua. Secondary giant cells arise as a result of the hypertrophy of the superficial cells of the träger and with the subsequent development of the placenta they form an integral portion of its superficial surface. Tertiary giant cells are found in the maternal tissue, appearing first in relationship to blood vessels and the intramyometrial connective tissue. They are most numerous in the antimesometrial region at the time of the greatest degeneration of the decidua capsularis: they degenerate slowly after parturition in the hamster. Orsini states that these tertiary giant cells are not likely to be confused with decidual cells because of their intravascular location, large size, time of appearance, and typical staining reactions. A further point stressed by Orsini in favour of the trophoblastic origin of these cells is the fact that when there is a large interval between adjacent gestation sites 'the invasive cell population declines as one passes from the periphery of a site to the interembryonic area'.

The binucleate cells in ruminants have a less complex cycle than those found in rodents; this is undoubtedly related to the type of implantation and placentation. They have been found in a large number of species (Assheton, 1906; Kolster, 1909; Andresen, 1927; Wimsatt, 1951; Björkman, 1954 are key references) including all the deer examined by us. Wimsatt (1950, 1951) and Björkman (1954) have provided a detailed history of these cells in the placenta of the sheep and cow. We would agree with Wimsatt (1951) who states 'the binucleate cells in different ruminants resemble one another morphologically, but there are indications that they are not completely equivalent functionally in all species'. He goes further and says that 'in many of their chemical and physiological attributes they resemble the syncytial trophoblast of the deciduate placenta and may with considerable justification be interpreted as the homologue of this tissue in the non-deciduate placentae of ruminants'. While not as invasive as those found in the human placenta (Boyd & Hamilton, unpublished), the binucleate cells of deer may well perform similar functions. Björkman points out that the terms 'binucleate' and 'diplokaryocyte' are not quite adequate, as the cells can sometimes be 'mono-' or 'polynucleate'. Irrespective of the number of nuclei, these cells are large with a high content of easily stainable granular cytoplasm. Although there have been other views (Kolster, 1909), modern investigators all agree that Assheton, Strahl and Andresen were correct in considering the binucleate cells to be trophoblastic in origin. Wimsatt (1951) finds that they do not make their appearance until about the 17th day of gestation. He states that they are formed by direct transformation of the columnar trophoblast through a gradation of stages into the typical definitive binucleate cells. Greenstein, Murray & Foley (1958) state that in the cow both binucleate cells and columnar trophoblast cells arise independently 'from a primitive polygonal "stem cell" which is characteristic of the early blastocyst'. We have insufficient early material to comment on this statement. Once the cell has matured into a typical binucleate cell, it apparently loses the power to reproduce itself. Increase in the number is probably brought about by transformation of primitive cells into further binucleate cells. The binucleate cells in sheep are especially numerous at the tips of the villi, less numerous along the sides and moderately plentiful between the bases of the arcades and in the membranous chorion; they remain in the same positions throughout pregnancy.

In the cow, Wimsatt found that binucleate cells are no more numerous at the tips of the villi than elsewhere, whereas Kolster (1909) finds them more numerous at the tips. We find no binucleate cells in the main-stem villi nor in the arcades between them in deer.

Many investigators have observed binucleate cells outside the chorion in several types of ruminants (Wimsatt, 1951). Assheton (1906) and Wimsatt (1950) found that in sheep the binucleate cells attach themselves to the denuded connective tissue of the crypt and form a primitive trophoblastic syncytium. In the cow Björkman (1954) states that binucleate cell formation is a property of both maternal and foetal tissues, but that interchange of binucleate cells from foetal and maternal epithelia may occur. Kolster (1909) observed binucleate cells passing from the trophoblast into the lining of the crypts in reindeer. The binucleate cells did not injure the lining and ultimately degenerated there. In *Dama*, binucleate cells pass from the trophoblast into the crypt lining from the time the villus has occupied a crypt. From then until term typical binucleate cells can be seen at intervals along the crypt lining. We have no convincing evidence that they give rise to the syncytial regions found in the crypt lining.

The function of the binucleate cells is still a matter for speculation. In sheep and in deer there is little doubt that on occasions the binucleate cells are phagocytic; some pigment granules derived from destroyed pigment cells are frequently found within them. It has been further suggested (Assheton, 1906, Kolster, 1909 and Andresen, 1927) that the binucleate cells produce a secretion which plays a part in destruction of uterine tissue. Wimsatt (1951, p. 259) comments that this is purely inferential since the cells do not possess pre-secretory granules. Björkman (1954) considers that they are responsible for the production of chorionic gonadotrophin and Greenstein *et al.* (1958) suggest their secretions may help to maintain the corpus luteum. Our electron micrographs show that giant cells lack well-developed microvilli and subjacent minute intracytoplasmic vesicles. This suggests that they are not primarily concerned with physiological exchange between mother and foetus.

SUMMARY

1. Details of patterns of reproduction are given for several species of Cervidae.

2. A description is given of implantation and of the development of the placentome in *Dama dama*, and of the appearances of the placentomes at certain stages of pregnancy in several other species of deer.

3. The morphological characteristics of the pedunculated placentomes including the arrangement of the villi are given; all species are oligocotyledonary.

4. Three zones are recognized in the villi and maternal septa. They are called zones of physiological exchange, attrition and storage.

5. The cytological and electron-microscopic characteristics of the trophoblast and crypt lining are described for the first time in Cervidae.

6. Trophoblastic and cryptal microvilli are well developed in *Dama* and small intracytoplasmic vesicles are pronounced in the apical regions of the trophoblast cells.

7. Binucleate giant cells are a constant feature of the trophoblast of the Cervidae. They contain a characteristic PAS-positive carbohydrate-protein complex. They are phagocytic; they lack microvilli.

8. The crypt lining in *Dama* consists of maternal cuboidal cells with apical microvilli, and in limited regions the lining is a syncytium also possessing microvilli. Binucleate cells, devoid of microvilli, are also included in the crypt lining. Remnants of dead cells are found in the foetal-maternal junction separating the microvilli of the two layers.

9. The uterine epithelium remains intact in the intercotyledonary region.

10. In most deer the tips of the maternal septa eventually undergo attrition to become atrophic filaments and the diaphthoro-epithelio-chorial condition prevails.

11. The foetal capillaries are arranged as an anastomosing circumferential plexus about the central artery and vein of the villus; many are intra-epithelial. The maternal capillaries form a trellis-work on the septal wall and are not intra-epithelial.

12. The origin and fate of the binucleate giant cells and the components of the crypt lining are discussed.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Plate 1

Photographs of the placentomes of various Cervidae. The umbilical vessels have been injected with coloured gelatin and the allantois has been opened in each specimen.

Fig. 1. Placentomes of a red deer (foetus 200 mm. c.r. length). $\times\frac{1}{4}$.

Fig. 2. Placentomes of a fallow deer (foetus 110 cm. c.r. length). $\times\frac{1}{6}$.

Fig. 3. Placentomes of a fallow deer (foetus 290 mm. c.r. length). Note the well-vascularized membranous chorion. $\times\frac{1}{8}$.

Fig. 4. Placentomes of a Japanese Sika deer (foetus 332 mm. c.r. length). $\times\frac{1}{8}$.

Fig. 5. Placentome of a Père David's deer (foetus 275 mm. c.r. length). $\times\frac{1}{3}$.

Plate 2

Fig. 6. Photograph of placentomes of a red deer pregnant with a 150 mm. c.r.-length foetus. The uterine vessels have been injected with yellow gelatin. About natural size.

Fig. 7. Photomicrograph of a caruncle of a fallow deer pregnant at 22 mm. showing early crypt formation. PAS and Light Green. × *c*. 3.

Fig. 8. Photomicrograph of another caruncle from the same specimen as above showing early crypt formation. H. and E. × 400.

Fig. 9. Photomicrograph of the tip of a developing villus within a shallow crypt in a caruncle of a fallow deer pregnant with a 27 mm. foetus. H. and E. × 600.

Fig. 10. Photomicrograph of the edge of another caruncle of the same specimen as above showing the continuity of the duct lining of an uterine gland with the caruncular epithelium. H. and E. × 400.

Plate 3

Fig. 11. Photomicrograph of the zone of attrition (page 11) of the placentome of a fallow deer (foetus 280 mm. c.r. length). H. and E. × 150.

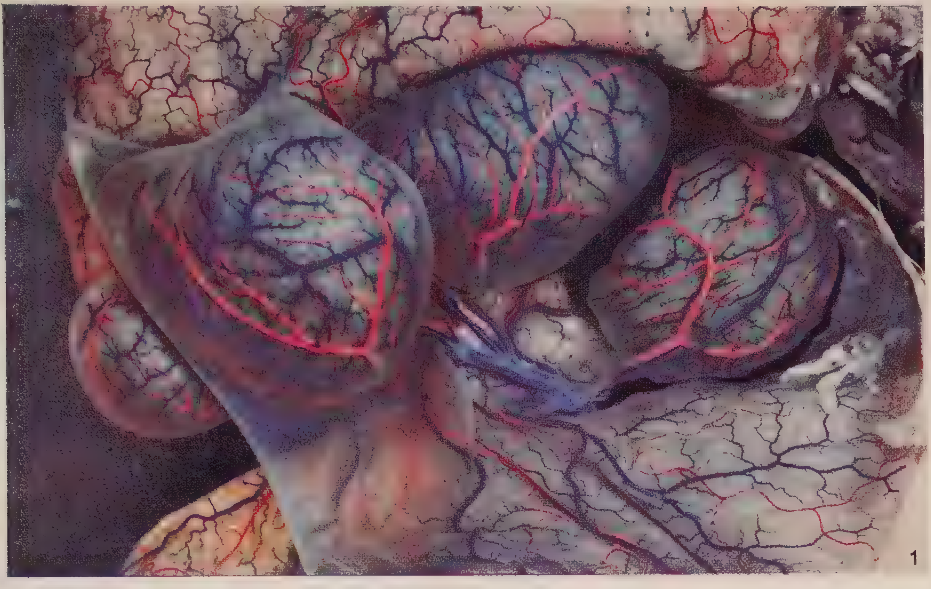
Fig. 12. Photomicrograph of a similar region of a placentome of the same animal as above showing glycogen in the columnar trophoblast cells of the main stem villi and the arcades. PAS and Light Green. × 180.

Fig. 13. Photomicrograph to show distribution of alkaline phosphatase (Na-β-glycerophosphate substrate) along the edge of the trophoblast and in maternal endothelial cells of the placentome of a fallow deer (foetus 190 mm. c.r. length). × 180.

Fig. 14. Photomicrograph of the columnar trophoblast cells of the main-stem villus (zone of storage, page 11) of the placentome of a fallow deer (foetus 220 mm. c.r. length). Compare with fig. 12. H. and E. × 500.

Fig. 15. Photomicrograph of the lower part of the zone of attrition (page 11) of the placentome of a fallow deer (foetus 180 mm. c.r. length). Binucleate cells are present in the trophoblast and in the crypt lining; syncytial masses are present on the right of the central maternal septum. H. and E. × 180.

Fig. 16. Photomicrograph of the tip of a villus in the placentome of a fallow deer (foetus 180 mm. c.r. length). H. and E. × 90.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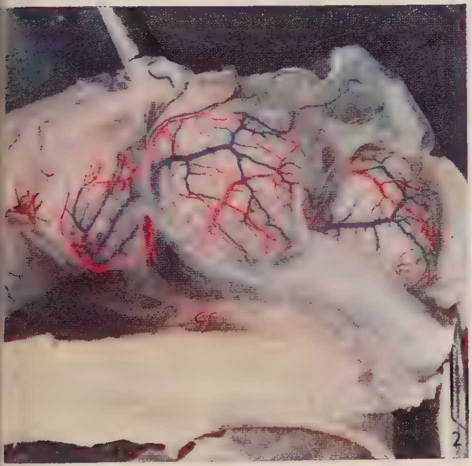
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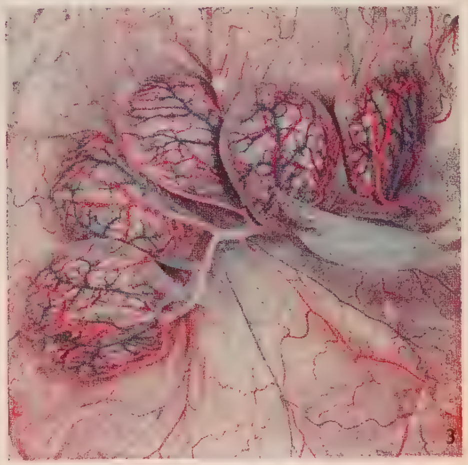
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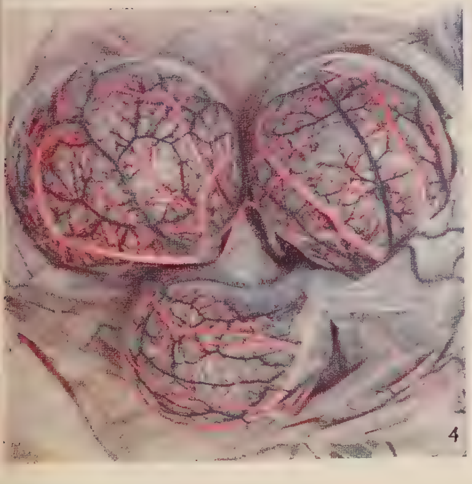
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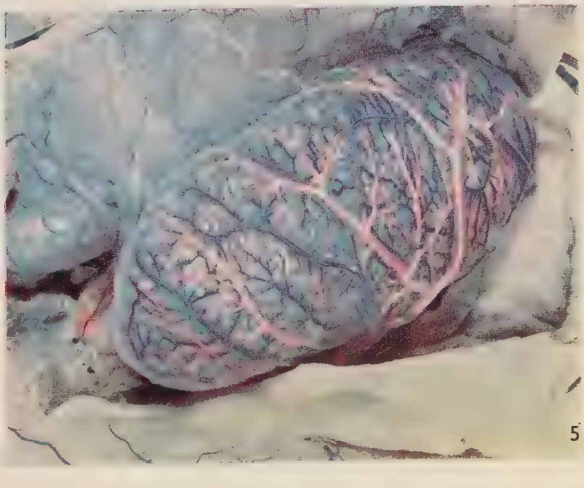
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HAMILTON, HARRISON AND YOUNG—ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE

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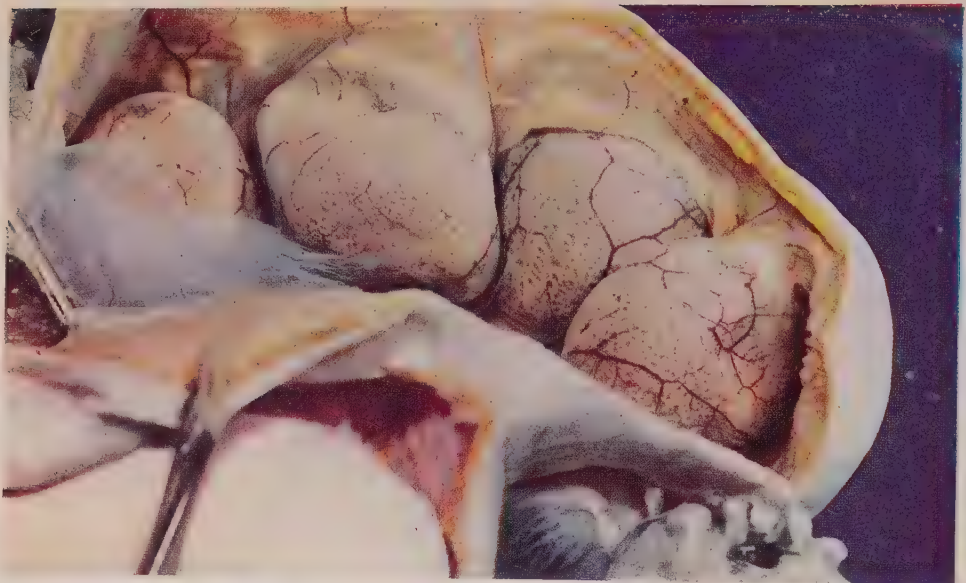

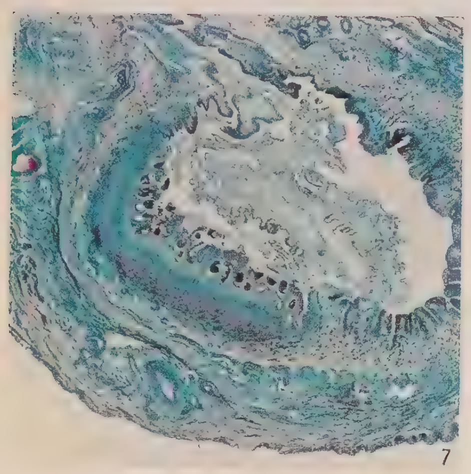

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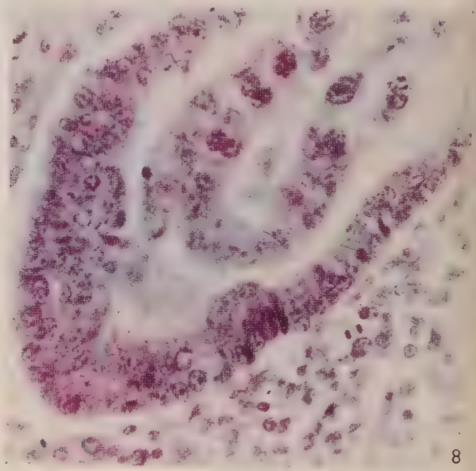

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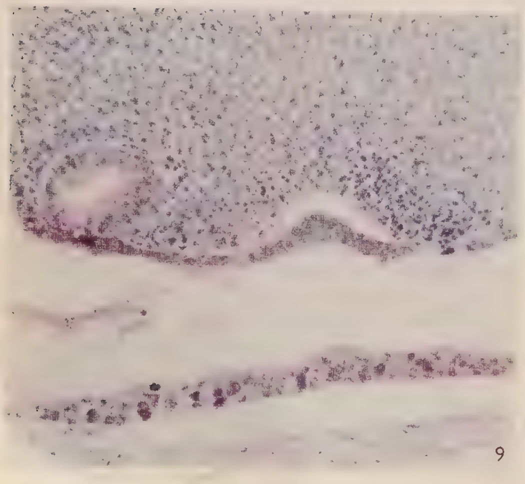

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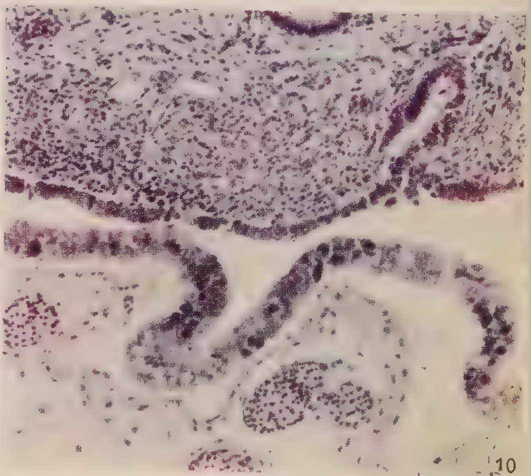

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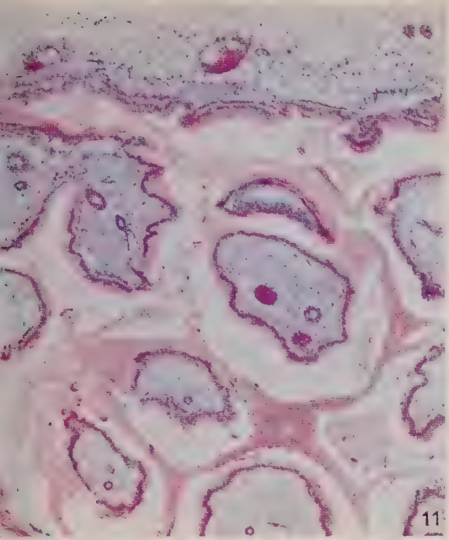
HAMILTON, HARRISON AND YOUNG—Aspects of placentation in certain cervidae



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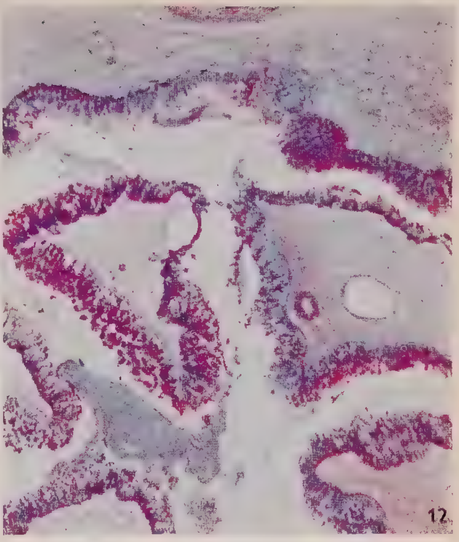

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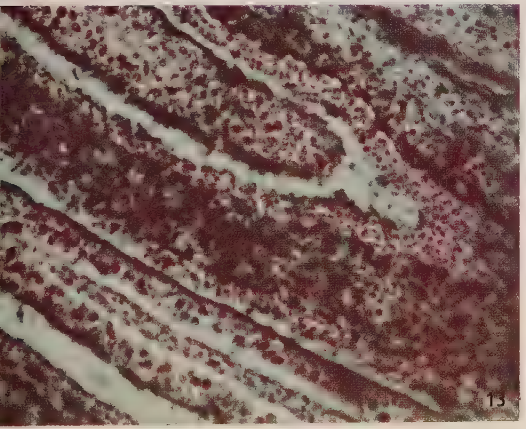

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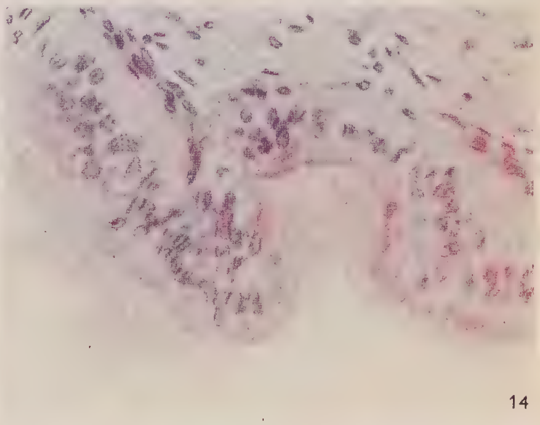

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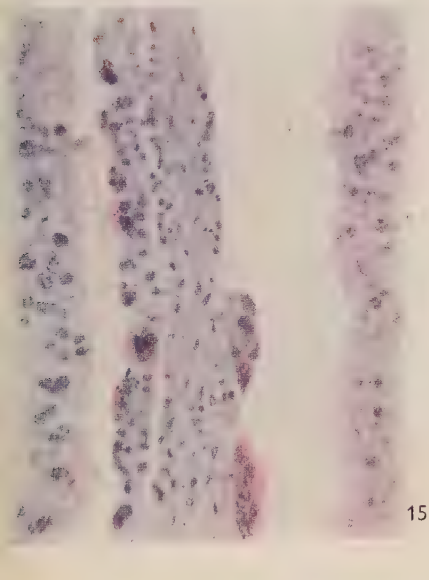

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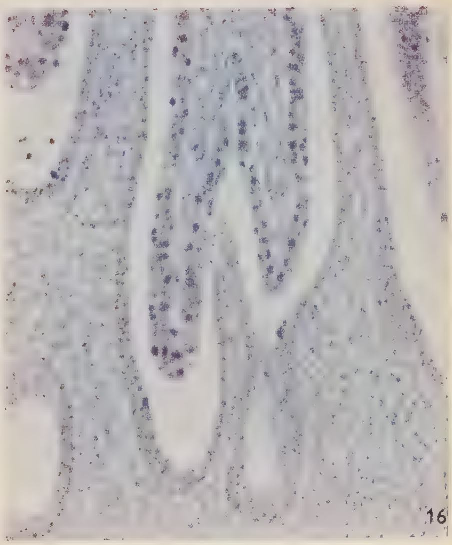

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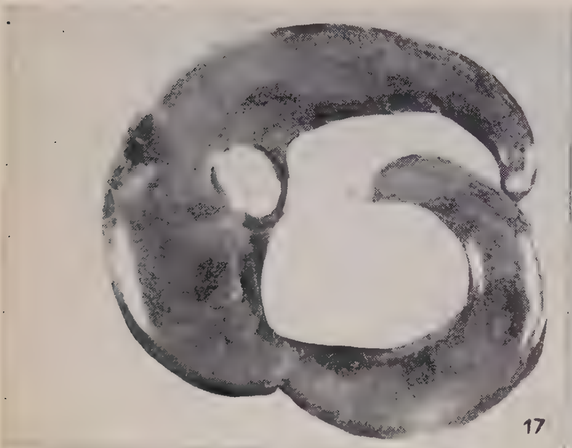
HAMILTON, HARRISON AND YOUNG—ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE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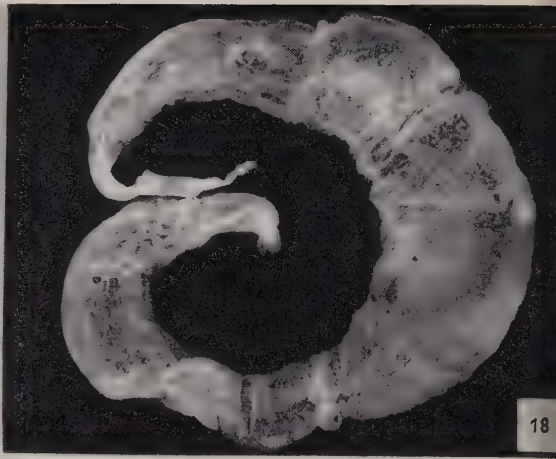
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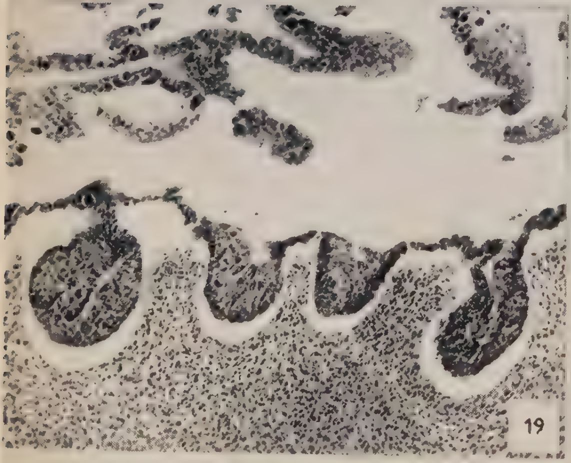
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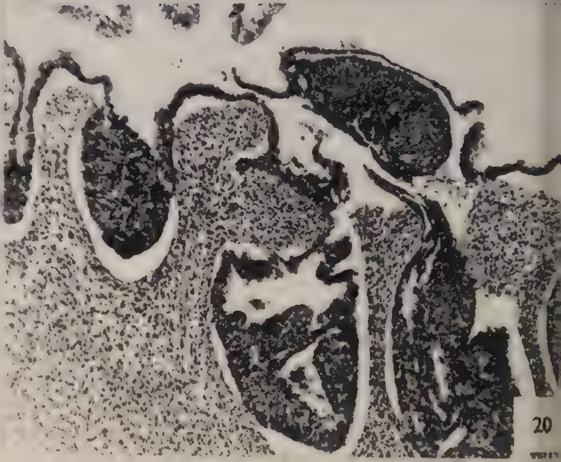
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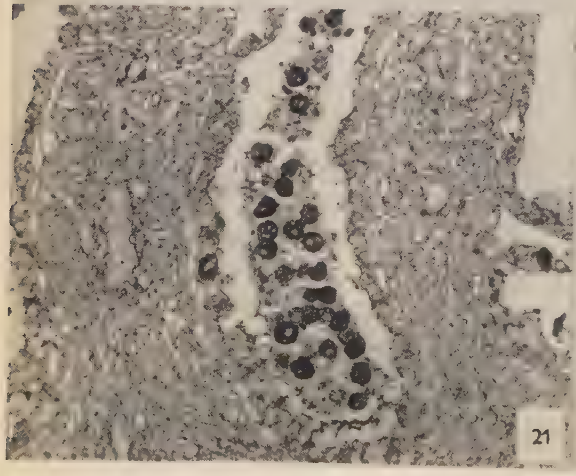
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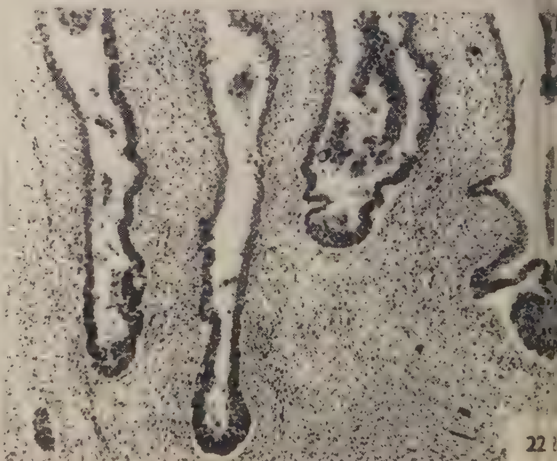
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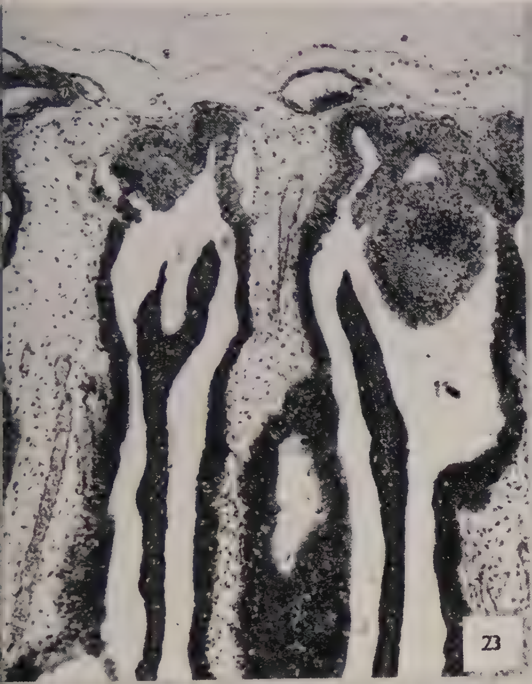
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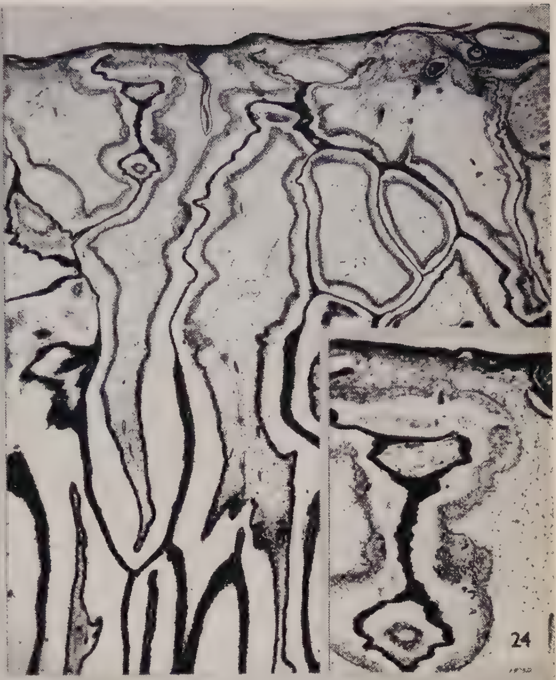
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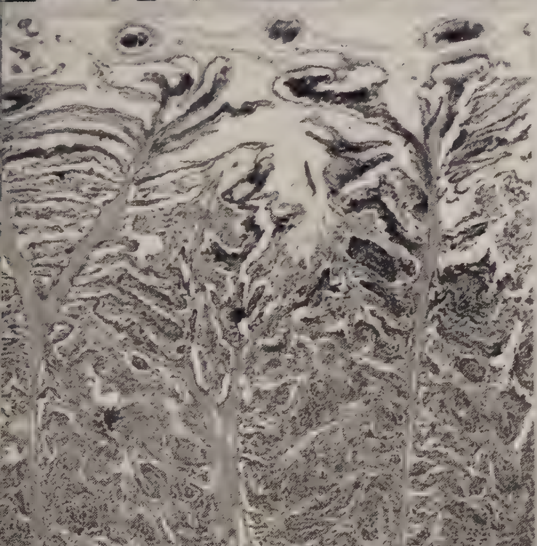
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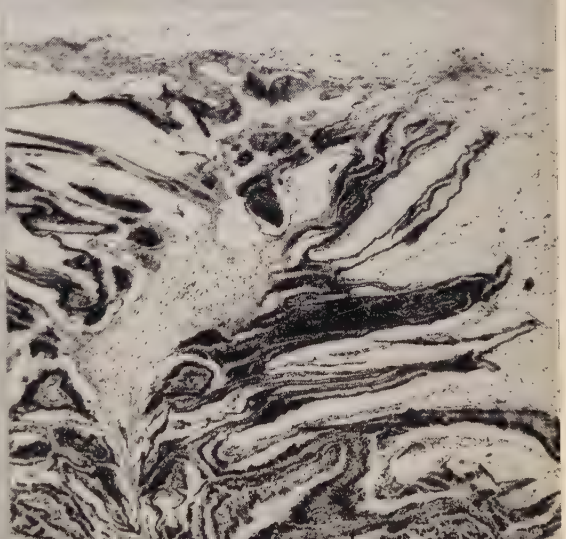
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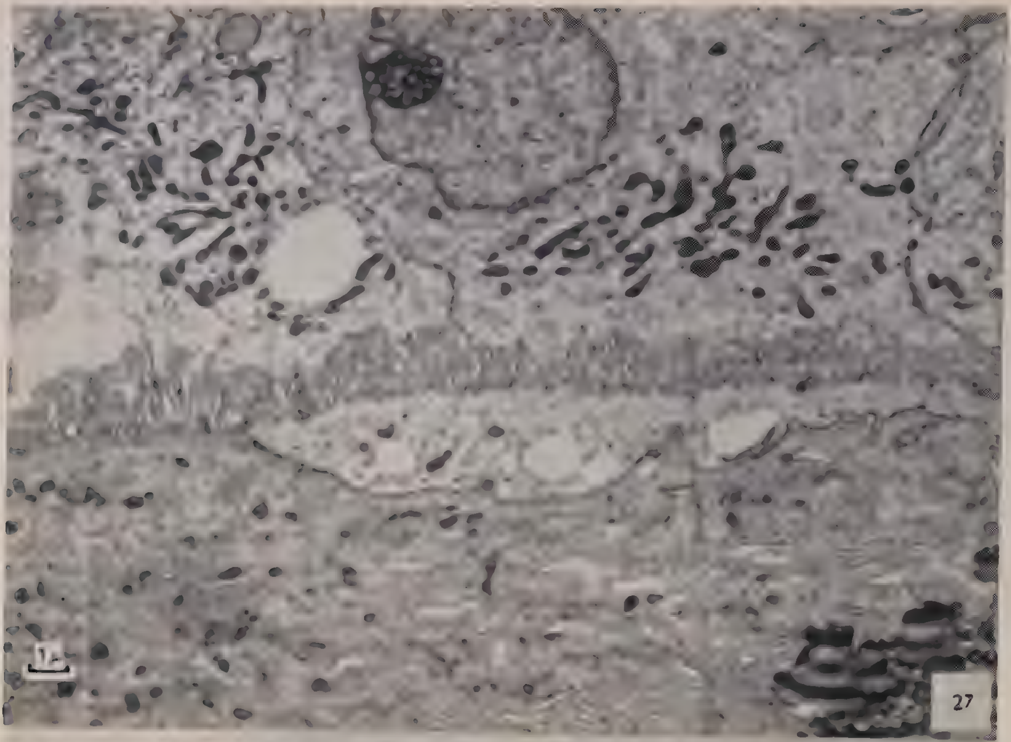

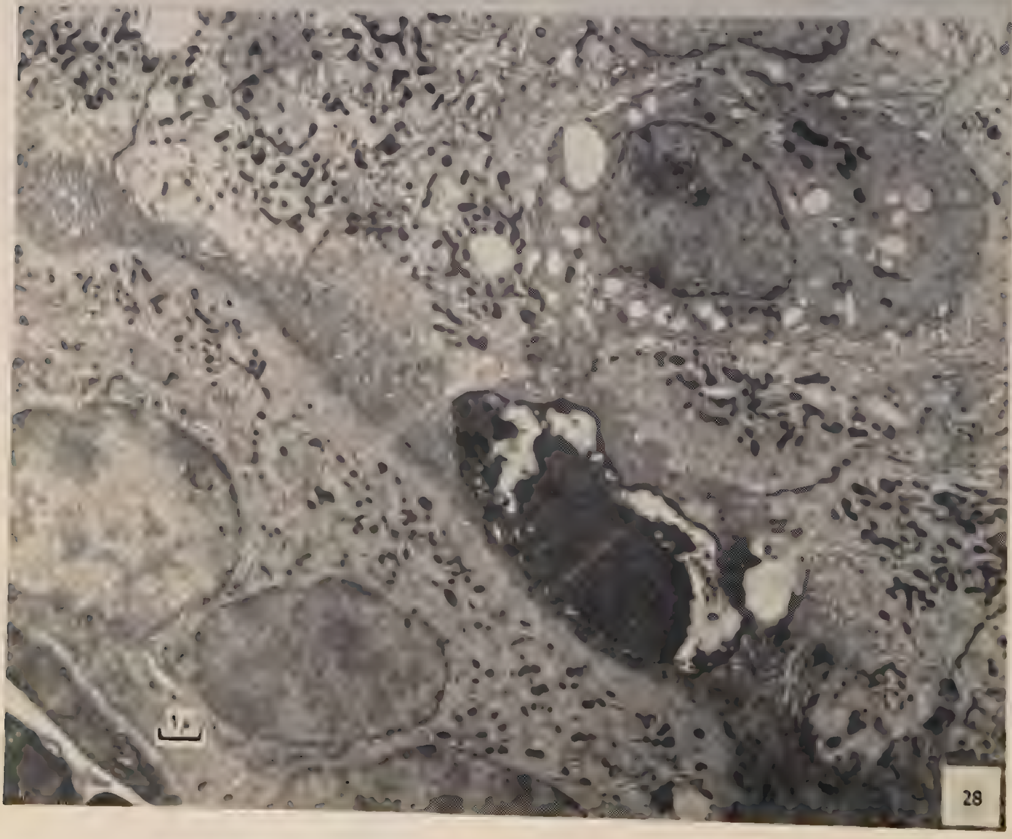

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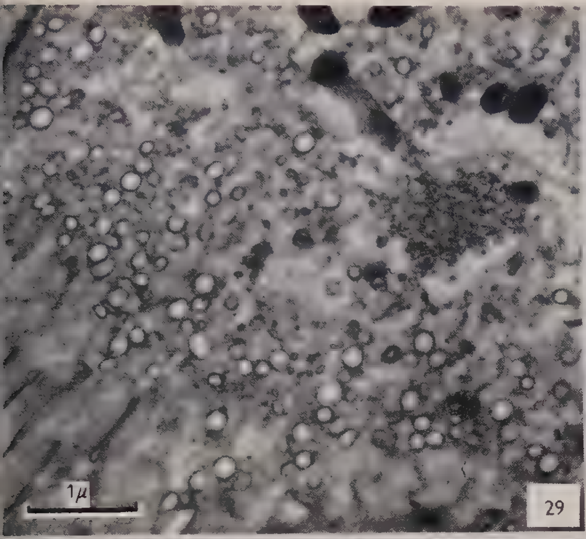

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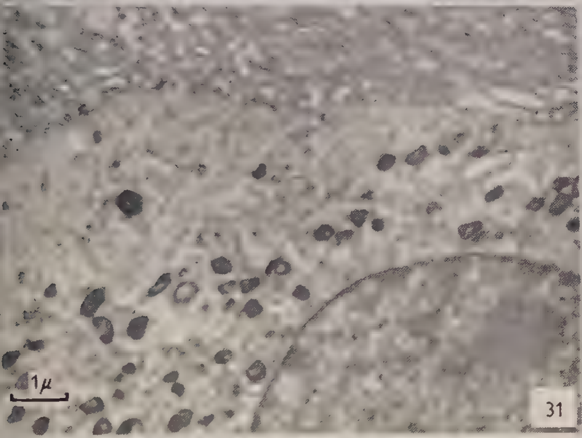

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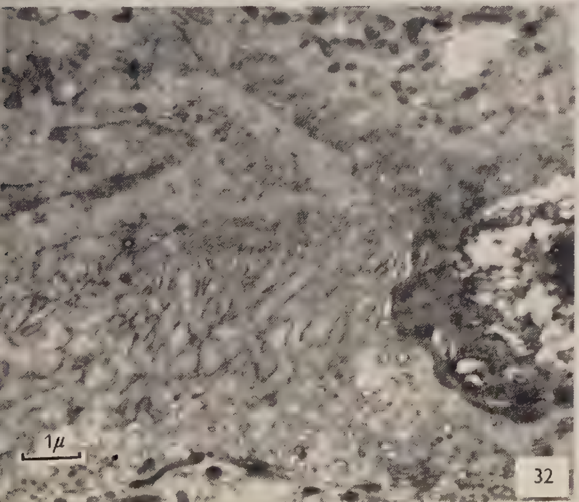

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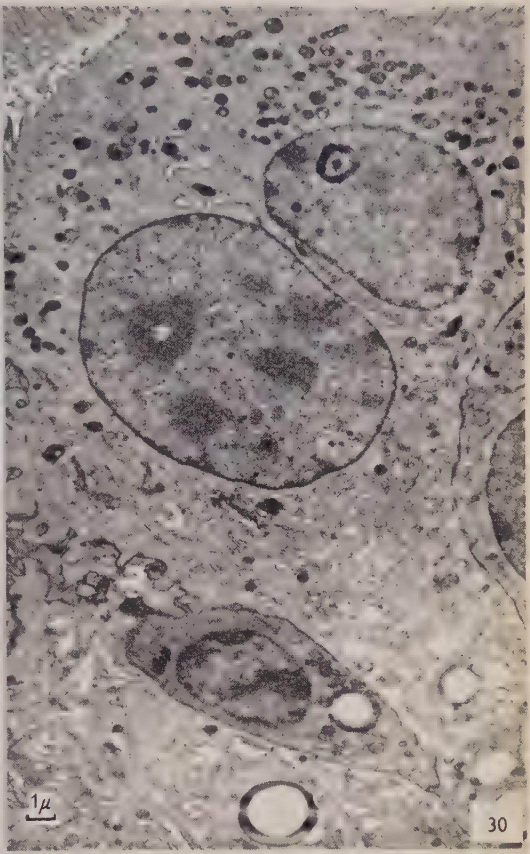

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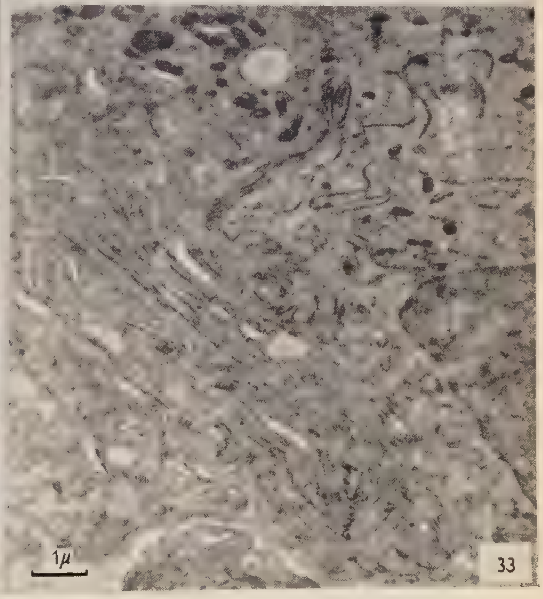

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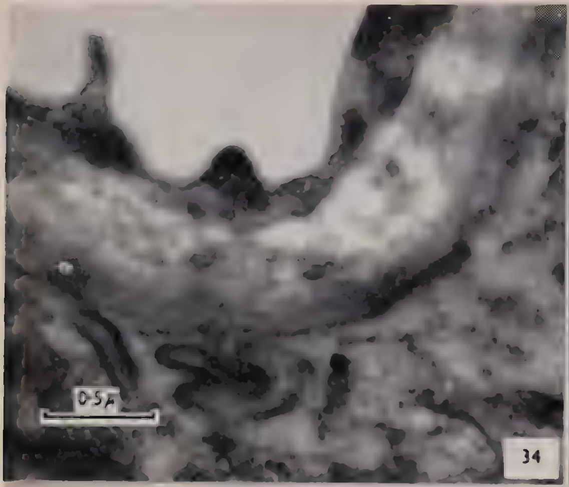

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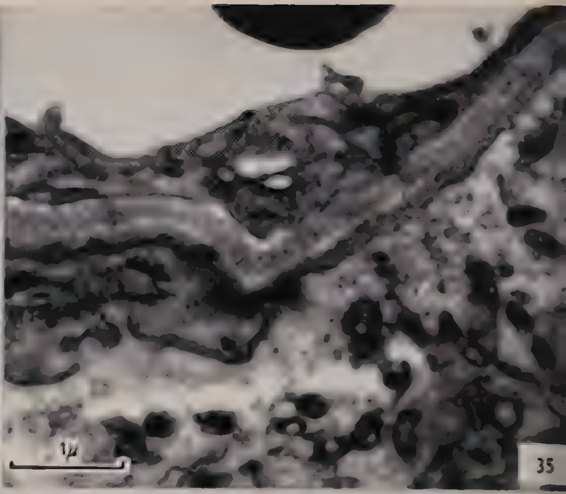

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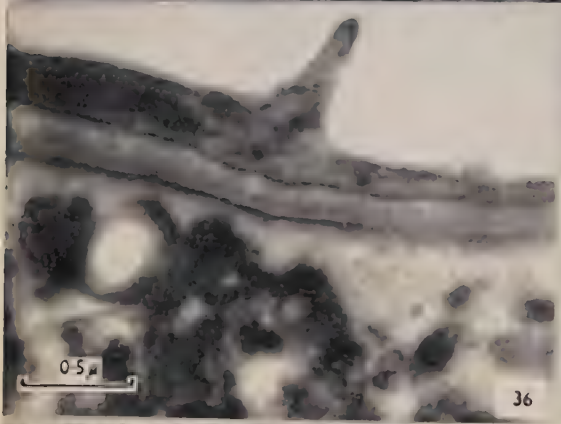

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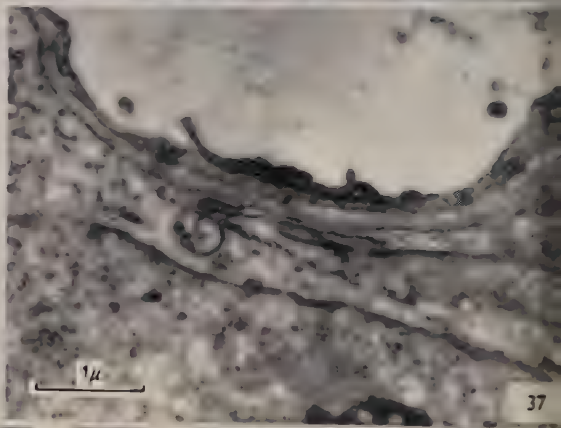

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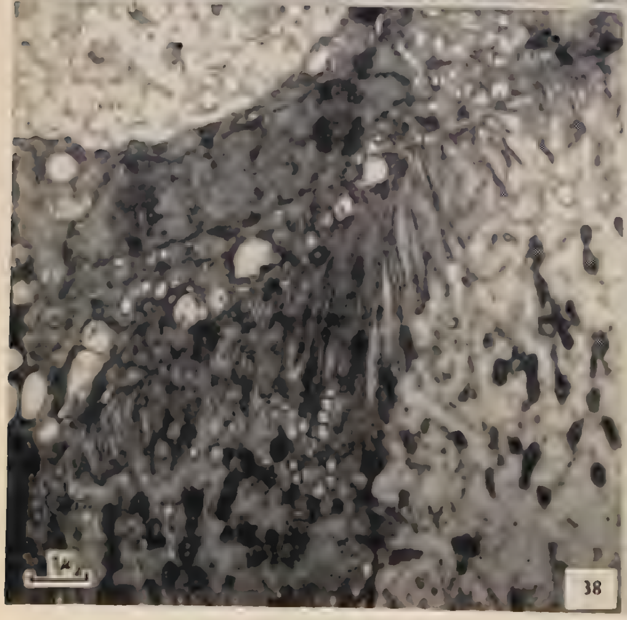

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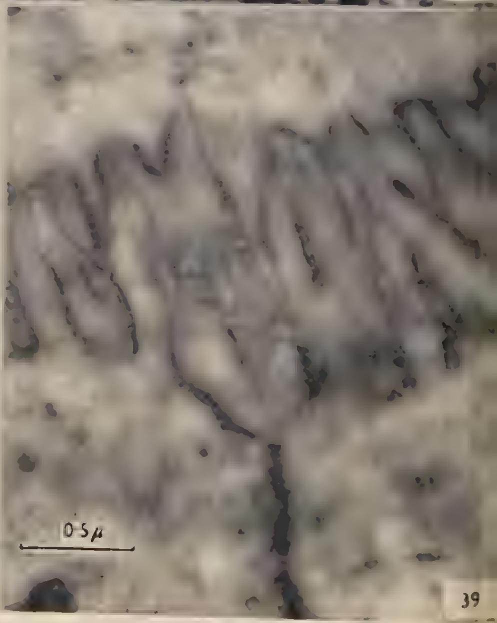

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PLATE 4

Fig. 17. Photograph of the chorionic sac of a fallow deer pregnant with a foetus 18 mm. in C.R. length. Note the developing cotyledons bearing short villi. × $\frac{1}{4}$.

Fig. 18. Photograph of the chorionic sac of a fallow deer pregnant with a foetus 25 mm. in C.R. length. Note atrophic tips to the sac. × $\frac{1}{4}$.

Fig. 19. Photomicrograph of a caruncle of a fallow deer pregnant with a foetus 12 mm. in C.R. length showing early crypt formation. H. and E. × 60.

Fig. 20. Photomicrograph to show a stage in crypt formation later than that shown above. The fallow deer foetus was 22 mm. in C.R. length. × 60.

Fig. 21. Photomicrograph of developing villus and crypt in the caruncle of a fallow deer 27 mm. in C.R. length. The section has been treated by the PAS technique which demonstrates the binucleate cells clearly. × 100.

Fig. 22. Photomicrograph of the developing crypts in the caruncle of a fallow deer pregnant with a foetus 33 mm. in C.R. length. Note the hypertrophied lining of the crypts' ends. H. and E. × 30.

PLATE 5

Fig. 23. Photomicrograph of the main stem villi and crypt mouth of a placentome of a roe deer (foetus 91 mm. in C.R. length). Compare the tips of the maternal septa with those shown in fig. 24. H. and E. × 60.

Fig. 24. Photomicrograph of the atrophic terminal portions of the maternal septa of a placentome of a fallow deer (foetus 290 mm. in C.R. length). The inset shows a portion of the tip of one septum enlarged. H. and E. × 30; inset × 60.

Fig. 25. Photomicrograph of part of the placentome of a reindeer (foetus 260 mm. C.R. length). H. and E. × 30.

Fig. 26. Photomicrograph of part of the placentome of a reindeer (foetus 290 mm. C.R. length). H. and E. × 50.

PLATE 6

Fig. 27. Electron micrograph of placentome of a fallow deer pregnant with a 200 mm. C.R. foetus, showing foetal-maternal junction. Foetal trophoblast cells above with line of mitochondria. Microvilli interlocking with maternal syncytium below. × 3900.

Fig. 28. As above showing foetal-maternal junction with included degenerating cell. Foetal trophoblast on the right and maternal syncytium on left. × 3900.

PLATE 7

Fig. 29. Electron micrograph of placentome of a fallow deer pregnant with a 200 mm. C.R.-length foetus, showing small vesicles at apex of foetal trophoblast cell. Microvilli on left. × 7350.

Fig. 30. As above showing maternal giant cell. × 1950.

Fig. 31. As above showing apex of maternal giant cell with few microvilli. × 3600.

Fig. 32. As above showing junctional zone with included dead cell on right. Small vessels and microvilli of foetal trophoblast cell below. × 3600.

Fig. 33. As above showing maternal crypt cell with endoplasmic reticulum and folded plasma membrane. × 3600.

PLATE 8

Fig. 34. Electron micrograph of placentome of a fallow deer pregnant with a 200 mm. C.R.-length foetus, showing maternal capillary and basement membrane. Lumen of capillary with process of endothelium above, cytoplasm and unfolded plasma membrane of crypt cell below. × 14700.

Fig. 35. As above showing maternal capillary and basement membrane. × 7350.

Fig. 36. As above showing foetal capillary and its basement membrane. Small process of foetal endothelium projecting into lumen of capillary. × 14700.

Fig. 37. As above showing apex of foetal capillary. × 7350.

Fig. 38. As above showing apex of foetal columnar trophoblast cell with microvilli, small vesicles and line of mitochondria. × 3600.

Fig. 39. As above showing interlocking of microvilli at foetal-maternal junction. Plasma membrane between two foetal trophoblast cells is seen. × 14700.

DEVELOPMENT AND STRUCTURE OF THE VILLOUS HAEMOCHORIAL PLACENTA OF THE NINE-BANDED ARMADILLO (*DASYPUS NOVEMCINCTUS*)

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INTRODUCTION

Chapman (1901) described the afterbirth of *Euphractus sexcinctus*, and Fernandez (1914) described implantation in *Dasypus hybridus* in some detail. Considerable attention was paid by the early workers to the form of the placenta regarding arrangement of the villi. Newman & Patterson (1910), in their paper on polyembryony, summarized the earlier studies concerning the structure of the placenta of *D. novemcinctus*, listed a number of different patterns of distribution of villi during the course of gestation, and suggested that these were modifications of a discoidal implantation. The most comprehensive description of placentation in the nine-banded armadillo is that of Patterson (1913). He described the early attachment of the blastocyst in the fundic tip of the uterus, yolk-sac inversion, germinal inversion, and the formation of the Träger. Both Fernandez (1909) and Patterson (1913) compared the early development of the armadillo placenta with the development of the rodent, referring specifically to the work of Melissinos (1907). Strahl (1913) pictured the villi as lying in part under the endometrium, but stated that the intervillous space was formed by erosion. In the work predating publication of Grosser's (1927) full development of his system of classification of placentas, little attention was paid to the relationship of the foetal vessels to the maternal blood vascular system. In their reviews of placental structure, both Mossman (1937) and Amoroso (1952) listed the placenta of *D. novemcinctus* as in the villous haemochorial class of Grosser. Mossman (1937), in summarizing the known information concerning the armadillo placenta, states that implantation is fundic, orientation fundic, amniogenesis by cavitation, shape modified discoidal, yolk-sac completely inverted, and that there is no decidua capsularis. Enders, Buchanan & Talmage (1958), in their study of the endometrium of pregnancy of the armadillo, reported that the cell columns and vascular villi invade the maternal blood sinuses which subsequently constitute the intervillous space.

The work reported here was initiated as a part of a comprehensive study of implantation in the armadillo (Buchanan, Enders & Talmage, 1956; Enders *et al.* 1958; Enders & Buchanan, 1959*a*, *b*). It was felt that the additional material available makes a study of placentation in the armadillo in the light of modern interpretations of placental function highly desirable.

MATERIALS AND METHODS

During the course of our research studies concerning implantation of the blastocyst, several hundred female armadillos have been examined. The majority of these animals were purchased from suppliers who caught them within 100-mile radius of Houston, Texas. Approximately half of the females examined were in some stage of pregnancy, predominantly the period of delay of implantation.

Histological preparations were made from the placental tissues of forty-seven females in all stages from the period of early implantation to parturition. Routinely, portions of the placentas were fixed in Zenker's and Carnoy's fluids and in buffered formalin. When better cytological preservation was desired, Flemming's fluid or Elftman's osmichrome fixation was employed. The fixed material was stained by haematoxylin and eosin or by a variant of Masson's trichrome mixture. Glycogen was demonstrated by the PAS method using diastase extraction as a control. Lipid was demonstrated by the use of Sudan Black B on formalin-fixed frozen sections. Where it was desired to demonstrate cytoplasmic basophilia, Zenker's or Carnoy's fixed tissues were stained in eosin and methylene blue at a pH of 5·6.

Most of the tissue was obtained from animals from the feral population. Estimation of the age of the conceptus had, therefore, to be made on the basis of the extent of development and size of the embryos. A number of specimens were obtained from animals in which implantation had been induced by bilateral ovariectomy (Buchanan *et al.* 1956). Implantation in these animals does not differ from normal implantation (Enders *et al.* 1958).

DESCRIPTION

The continuous series of morphological changes in the formation and growth of the armadillo placenta can be divided for convenience of description into three stages. The first of these stages constitutes the period of placental establishment, beginning with the attachment of the blastocyst to the endometrium and ending with the formation of the cell columns. The second stage is that of placental enlargement, starting with the formation of the vascular villi and ending with the disappearance of the cytotrophoblast. The final stage, extending from the disappearance of the cytotrophoblast until parturition, is the period of the mature placenta.

Period of establishment of the placenta

Observations on the few previllous stages we have correspond well to Patterson's (1913) description of early implantation. The salient features in initial implantation which have been described by Patterson will be summarized in order to provide the necessary background for the interpretation of later stages. The initial attachment of the blastocyst is afforded by the cells of the trophoblast overlying the embryonic cell mass (Rauber's layer). After attachment has been initiated, the abembryonic trophoblast degenerates. At this time, the endoderm has extended only slightly beyond the margins of the embryonic cell mass. Consequently, an inverted yolk-sac placenta is formed without there being a bilaminar omphalopleure stage. The extraembryonic space forms initially between the embryonic cell mass and the overlying trophoblast. The mesenchyme therefore arises from trophoblastic cells. As implantation progresses, the trophoblast becomes thickened marginally and reduced over the initial site of implantation. These changes, reported by Patterson (1913), constitute the initial implantation. They are probably completed within the first 2 or 3 days of implantation, as judged by the rarity of these stages in our collection and by estimation of the date of implantation from the animals in which early implantation was produced by bilateral ovariectomy.

The avillous placenta, consisting of the inverted yolk-sac placenta and the trophoblast, is formed by the developments summarized above. The placenta persists in this form during the budding of the ectodermal plate and subsequent division into four individuals of the intraembryonic portion of the conceptus. An example of this stage from our specimens is illustrated in Pl. 1, fig. 1. At the periphery of the implantation site the trophoblast forms a thick ring. The endoderm of the yolk-sac is continuous with the luminal margin of this ring. Extending radially into the endometrium are tongues of trophoblastic tissue. This trophoblast appears to be partially syncytial and is vacuolated in paraffin preparations. It is only here in the peripheral region that the trophoblast is interdigitated with maternal tissues. A few slips of this invasive trophoblast may occasionally be found more centrally. The inner aspect of the peripheral ring of trophoblast consists of a mass of relatively cuboidal cells in which mitotic figures are numerous. Extending centrally over the implantation site and separated from the newly formed amnion by the developing exocoelom is a layer of syncytial trophoblast with altogether different characteristics than those of the invasive peripheral syncytium. This central syncytium resembles the epithelium which later clothes the vascular villi.

The inverted yolk-sac placenta consists of a layer of endodermal cells and a single layer of squamous mesenchymal cells. It forms a continuous sheet over the undivided ectodermal plate and separates the rest of the conceptus from the uterine lumen. At this stage the intraembryonic portions of the endoderm do not differ histologically from the cells of the extraembryonic portion.

The maternal tissues underlying the central trophoblast show evidence of deterioration. Even in the two earliest specimens which we have examined, the maternal blood sinuses have been tapped. (The blood sinuses lie particularly close to the surface in the fundic region where the blastocyst implants (Enders *et al.* 1958). More peripherally, where the tongues of trophoblast extend into the maternal tissues, the endometrium is less disorganized.

Period of placental expansion

(1) *Initial formation of villi*

The embryos from the specimens which we obtained in this stage constitute a series from primitive streak formation to early somite development. In the earliest specimens only scant evidence of incipient haemopoiesis is seen in the splanchnopleure. In the later specimens, numerous normoblasts fill the lumina of the well-developed vessels of the splanchnopleure. The diameter of the implantation site and the extent of erosion of maternal tissues varies considerably. In all our specimens, however, the maternal sinuses have been interrupted but not invaded.

The peripheral trophoblast displays essentially the same characteristics as in the previous stage (Pl. 1, figs. 2, 3). The central trophoblast, however, is quite different.

It is much more extensive than previously and exhibits, in addition to the syncytial epithelium, masses of cellular trophoblast extending from the surface (Pl. 1, fig. 2; Pl. 2, figs. 7, 8). When first seen, the cell columns (Träger's knots) are in isolated patches of only a few cells (Pl. 2, fig. 7). Each cluster of cells is covered by an especially thin layer of syncytium which is continuous with that of the underlying central trophoblast. In specimens in which cell columns are more numerous, areas may be found in which some of the cell columns are so close together that on first examination they appear to form a continuous multilayered portion of the trophoblast.

As the cell columns enlarge, they protrude from the surface to a greater extent (Pl. 2, fig. 8). In the late stages of cell-column formation the appearance is one of knobs of cells covered by a thin layer of syncytium located on the surface of a membrane consisting of syncytial trophoblast and several layers of mesenchymal cells. This latter membrane, which has been derived from the central trophoblast, is the chorionic plate. Evidence of blood-vessel formation is seen in the associated mesenchyme. The chorionic plate at this stage may be applied fairly closely to the maternal tissues, but more frequently it forms pockets containing extravasated maternal blood.

(2) *Formation of vascular villi and invasion of sinuses*

The first sign of the development of the vascular villi is the protrusion from the surface of the chorionic plate of small folds of tissue having the same morphological make-up as the chorion itself but capped by a cell column. These vascular villi are simple projections covered by syncytial trophoblast with a mass of cytotrophoblast at the distal extreme. Initially the exocoelom may extend partially into the villi. With continued development the villi become highly branched and the exocoelom is excluded from these structures.

In the early stages of their development the vascular villi are situated in a pool formed by extravasation of blood from the interruption of the endometrial blood sinuses. By the late limb bud stage, the villi have invaded the endometrial sinuses peripheral to the initial implantation site (Pl. 1, fig. 4). The villi subsequently invade the entire anastomotic system of basal endometrial sinuses. Progressive stages exhibit both extension of villi toward the cervix and the enlargement of the sinuses. Maximum expansion of the maternal blood sinuses is usually observed by the time the foetuses have reached a length of 5 cm.

The endothelium of the maternal sinuses remains intact except in the area of initial penetration (Pl. 3, fig. 13). Enlargement of the intervillous space is by passive stretching of the endometrium by the growing villi. It should be emphasized that in no instance has evidence been observed that the cell columns or vascular villi are erosive or actively invasive of the endometrial tissues *per se*.

Although the villi develop first in the centre of the implantation area, later the chorion covering the fundic tip of the uterus becomes relatively avillous. Four major villous areas develop corresponding to the quadrants of the uterus. The foetal vessels enter these areas near their fundic margin. The villi within these continuous areas exhibit extensive arborization, anastomosis and short non-anastomotic terminal projections. In younger placentas the terminal portions of the villi are plate- or frond-like expansions (Pl. 2, fig. 11). Foetal capillaries form a radiating series of loops within the vascular portions of each projection, while the distal margin is formed by a crescentric cell column. In later placentas those terminal portions of the villi which do not anastomose are simple finger-like projections (Pl. 2, fig. 12).

When the body stalks of the embryos are formed, the epithelium of the inverted yolk-sac is separated from the entoderm of the embryos, except for a transient yolk stalk. The inverted yolk-sac constitutes the outer covering of the embryonic vesicle. In other words, the four developing embryos with their amnions are contained in an exocoelom, the splanchnopleure of which is covered by the endoderm of the yolk-sac. In specimens from early in the period of placental expansion, the inverted yolk-sac placenta is highly vascularized (Pl. 1, fig. 4). The epithelial cells are tall columnar and frequently exhibit both vacuolation and granulation in the distal cytoplasm (Pl. 1, fig. 5). However, prior to the disappearance of the cell columns, the nature of the epithelium of the yolk-sac alters. The cells become more cuboidal and, in places, stratified, and the underlying connective tissue becomes less vascular and more dense. The yolk-sac placenta of foetuses of around 3·5 cm. is relatively avascular.

The peripheral ring of trophoblast remains as the area of junction between the endometrium and the placenta. Relatively little evidence of invasion or proliferation can be seen here in the later aspects of this stage. At the junction between the yolk-sac and the trophoblast, a roll of luminal epithelial cells is frequently seen (Pl. 1, fig. 4). By the end of the period of expansion fusion of the overlying placental tissues with the luminal surface of the endometrium has been initiated and the peripheral ring is no longer distinguishable as such.

Mature placenta

By the time the foetuses have reached a crown rump length of 6 or 7 cm. the mature placenta has been formed. This stage persists until parturition (neonatal length 12 cm). Cell columns are absent, and it is doubtful that the placenta is increasing in size. The definitive position of the placenta in the uterus has been reached. The portion of the chorion overlying the fundus is relatively devoid of villi, but they are numerous in the sinuses of the body of the uterus (Pl. 2, fig. 6). The villi are highly branched, anastomotic structures, forming a meshwork composed of looping, branching chords of a relatively uniform diameter. The villi are massed in the four quarters of the uterus, while the four foetuses lie dorsal, ventral, left and right.

As previously stated, the umbilical vessels to each quarter of the continuous placenta are situated at the fundic end of the more villous portion. In addition, the vessels enter and leave relatively close to the overlying endometrial tissue. In other words, few villi are present on the side of the vessels directly underlying the endometrial arcades but numerous villi extend towards the muscularis. The blood flows via the umbilical arteries into a capillary bed extending in a highly tortuous fashion toward the inner muscular layer of the endometrium, and then back towards the luminal surface before confluence into a number of veins leading toward the umbilical vein at the fundic end of the placenta. It should be noted that maternal blood flow is still in the opposite direction; that is, maternal blood enters the luminal side of the sinuses from vessels in the arcades of endometrium. The return flow is through the spongework created by the villi and into the numerous smaller sinuses in the inner layer of the myometrium.

In the mature placenta the endometrium is highly stretched, glands are few and poorly organized. The major change that occurs during this period is the fusion of the extraembryomic membranes overlying the endometrial arches with the now reduced endometrial tissue. By the time the foetuses have reached a C.R. length of 8 cm. fusion has occurred as far as the cervix.

Histology and histochemistry of the villi

The trophoblastic cell columns consist of aggregates of similar cells, the cytoplasm of which is slightly basophilic. Throughout most of their existence, the columns are characterized by the presence of numerous mitotic figures. Small lipid droplets are a conspicuous feature of many of the cells in the cell column (Pl. 3, fig. 20). Glycogen is only rarely present. Surrounding the cell columns is found a thin layer of syncytial trophoblast (Pl. 3, figs. 13, 18). This trophoblast has a more uniform free surface than has the epithelium of the vascular villi with which it is continuous. In most instances the cell columns appear to be completely surrounded by syncytium, but it is occasionally too thin to be readily discernible with the light microscope. No basement membrane was observed between the cells of the cell column and the syncytium. There are numerous intercellular spaces within the columns. A PAS positive material which resists diastase extraction is present in most of these spaces (Pl. 3, fig. 18). This material is granular, and only mildly acidophilic.

The vascular villi change appreciably in morphology during the course of gestation. In all stages, however, they are characterized by the presence of a single layer of syncytial epithelium and the complete absence of any cytotrophoblast. The surface of the syncytium is very irregular, exhibiting a brush border in some areas and a relatively smooth surface in other areas (Pl. 3, figs. 15–17). Frequently small vacuoles are found within the syncytium; occasionally large vacuoles are seen. Areas of the syncytium that exhibit protruding masses of cytoplasm are common. These cytoplasmic protrusions have a seemingly tenuous connexion with the syncytium (Pl. 3, fig. 17). At no stage of gestation does the syncytium contain appreciable amounts of demonstrable lipid. Cytoplasmic basophilia is characteristic of the mid-zone of the syncytium; that is, especially around the nuclei. The syncytium covering the vascular villi is underlain by a basement membrane of variable thickness (Pl. 3, fig. 16). This membrane is quite thin during the early expansion period of the placenta, but grows progressively thicker. The membrane is PAS positive, diastase resistant and acidophilic. The mesodermal core of these villi consists of the foetal capillaries and numerous fibroblasts derived from the mesenchymal cells. Throughout the period of expansion of the placenta the connective tissue is relatively loose (Pl. 2, fig. 9). The foetal capillaries are situated more or less randomly, although there is some tendency toward a peripheral location. Lipid is present in the perivascular cells (Pl. 3, fig. 20). Glycogen is occasionally present, especially in cross-sections of the larger villi (presumably nearer to the chorionic plate) (Pl. 3, fig. 19).

In late pregnancy after the disappearance of the cell columns, the structure of the villi is somewhat different. The syncytium is generally thinner, although its surface exhibits almost as much variability as at earlier stages (Pl. 3, fig. 14). There are fewer nuclei per unit area. The syncytium is thickest at the bulges where the nuclei are located. The juxtanuclear area of the syncytium remains slightly basophilic. The basement membrane underlying the syncytium is thick, and contains numerous collagen fibres as well as PAS positive material. The stroma of the villi is more fibrous than previously. The foetal capillaries lie close to the syncytium, but do not indent it (Pl. 2, fig. 10). The fibroblasts in striking contrast to the previous stage have become rounded, gland-like cells (Pl. 3, fig. 14). They exhibit pronounced basophilia in the cytoplasm which surrounds the Golgi zone.

In addition to the healthy tissue, degenerating villi are common in late pregnancy. Both fibrin and fibrinoid, as judged by the criteria of Wislocki & Bennet (1943), are found in these areas. Fibrinoid is especially common.

DISCUSSION

The invasion of the maternal blood sinuses and the enlargement of these sinuses by the villi to form the intervillous space is a unique feature of placentation in the armadillo. The significance of this method of development is readily apparent. Since the maternal vascular system is uninterrupted except in the fundic area, the blood flow is unidirectional (Enders *et al.* 1958). The maternal blood enters the sinuses under the arches of endometrial tissue. The flow is then toward the inner muscularis. This pattern of blood flow should result in fewer areas of relative mixing of oxygenated and deoxygenated blood than are found in the intervillous spaces of the human and monkey placentas, in which the blood both enters and leaves from the basal plate (Boyd, 1956; Ramsey, 1959). The foetal blood vessels also enter the intervillous space near to the luminal border of the placenta. Thus the venous return flows from the muscularis side of the placenta to the luminal side. In consequence, there is in all probability a rough countercurrent with the more oxygenated blood in the foetal vessels being near the luminal surface. There would not, however, be a direct countercurrent since the highly branched and anastomotic villi form a rather complete spongework in the maternal sinuses. In any given capillary therefore, the direction of foetal blood flow could bear any relationship to the direction of maternal blood flow. Although the means of formation of the intervillous space is quite different, the pattern of maternal blood flow in these spaces is similar to that of the villous haemochorial placenta of the hyena (*Crocuta crocuta*) as described by Amoroso (1959). The disappearance of villi from the fundic region results in the superficial alteration of the placenta from a discoidal one to a relatively zonary type. More important, it means that the villi disappear in the one area where the maternal blood vessels are not preserved because of their destruction during the initial penetration of the trophoblast.

The yolk-sac epithelium alters in character about the middle of the period of expansion of the placenta. Since, at this time, it constitutes the separation between the uterus and the splanchnopleure of the exocoelom, it is likely that this is the placental membrane which fuses with the surface of the endometrium in later stages. An examination of the mature placenta, however, reveals very little epithelium covering the connective tissue that is fused with the endometrium. Furthermore, what little epithelium is present has a somewhat different character from that of the earlier yolk-sac epithelium. An alternative method of development would be the outgrowth of the chorion from the junctional zone to fuse with the endometrium and isolate the yolk-sac. Further studies will be necessary to clarify the morphology of the yolk-sac in the later stages of pregnancy.

The early villi have frond-like terminal projections. The villous trees observed in later pregnancy are composed of anastomotic cylindrical chords and terminal projections. There are, however, discontinuities in the early projections. The fusion and perforation of the syncytial trophoblast might be the means by which the plate-like initial terminations are converted to a series of anastomosing cylinders. An additional modification which would contribute to the change in form of the villi is the break-up of the trophoblastic cell columns capping the projections into smaller masses of cells (cell islands).

During the period of establishment of the placenta the peripheral ring of trophoblast apparently acts as the proliferative area. Mitotic figures are numerous there. The few cells which penetrate into the endometrium become highly modified and seem to degenerate. The more centrally located trophoblast appears to be formed by additions from the peripheral area. Consequently, the peripheral ring serves as a source of the trophoblast in general, a source of invasive tissue, the region of attachment to the uterus, and the junction point of the yolk-sac and the trophoblast. The area in which the trophoblast is actually invading the endometrium is the only true 'junctional zone'. Although this area of close relationship between the endometrium and the placenta persists into the period of placental expansion, active destruction of the endometrium occurs only in the early stages of the period of placental establishment.

In the armadillo placenta the only cytotrophoblast present after formation of the vascular villi is that of the cell columns. The absence of Langhans cells, of anchoring villi, and of basal plate simplifies the interpretation of the function of the cytotrophoblast in this species. From a structural point of view, the role of the cytotrophoblast is apparently to act as a source of proliferation of syncytial trophoblast. Both the restriction of mitotic figures to the cell columns and the position of these columns indicate that these structures constitute the proliferating tips of the growing villi. It is also possible that the cytotrophoblast may have a glandular function, as evidenced by the presence of intercellular, PAS positive material. Before a glandular function can be ascertained with certainty, however, evidence for liberation of the intercellular material must be accumulated. It is interesting to note that the staining qualities of this intercellular substance are those which would be expected of a gonadotropin.

Discussion of the functional activity of the armadillo placenta is premature at this time. However, a few inferences may be drawn from the observations to date. The strictly limited active invasion of the endometrium by the trophoblast suggests that the products of histolysis are of relatively little importance to the developing conceptus. The early degeneration of the abembryonic trophoderm produces an inverted yolk-sac placenta that is admirably situated to receive the products of glandular secretion. Penetration of the maternal sinuses also occurs very early, when the embryos would probably have a relatively limited capacity for synthesis.

On the other hand, the formation of the intervillous spaces by invasion of a pre-existing system not only leaves these spaces with a directional blood flow, but also causes relatively little interference with the vascular supply to the overlying endometrial glands. Consequently the products of the glands, probably garnered by the yolk-sac, would appear to have greater importance to the developing conceptus of the armadillo than to other species with a placenta of the villous haemochorial type. However, Boyd (1959) recently pointed out that the products of glandular secretion (histopoietic histototrophe) are probably more important to the early human embryo than has been generally recognized.

SUMMARY

Placentation in the armadillo was studied with special attention to the formation, development, and structure of the villi. Placental development can be divided into three stages: the period of establishment of the placenta, the period of placental enlargement, and the period of the mature placenta. Initial attachment, formation of the inverted yolk-sac, and the formation of the avillous placenta occur during the first period. Formation of cell columns and vascular villi, and the invasion of the maternal blood sinuses, occur during the second period. By the time the third period is reached, the blood sinuses have been invaded throughout the body of the uterus. There are no cell columns present in mature placentas, and no evidence that growth takes place during this period. The definitive form of the placenta is a spongework consisting of anastomotic and highly branched villi situated within the maternal blood sinuses.

The cytotrophoblast is composed of the cell columns (and islands) forming the distal ends of the villi. Neither Langhans cells nor anchoring cell columns are present, nor is there a basal plate at any stage. The cell columns are composed of aggregates of cells, many of which exhibit mitotic figures. Small lipid droplets are present in many of these cells and a proteinaceous, PAS positive substance is present in the intercellular spaces. The cell columns are invested by a thin layer of syncytium. The vascular villi in the early stages are frond- or plate-like extensions from the villous stem. The syncytial trophoblast covering the vascular villi has numerous and varied modifications of its free surface, and is underlain by a basement membrane. Numerous capillaries are seen in the stroma of the villi. In later stages the villi are composed of anastomotic strands with a relatively uniform diameter. The syncytium is thinner than at early stages and there is a great deal of collagen in the stroma. The foetal capillaries lie close to the syncytium but do not indent it. There are numerous highly modified fibroblasts with markedly basophilic cytoplasm within the villi.

The structure of the villi, position of the cell columns, and restriction of mitotic figures to the cell columns lead to the conclusion that these structures constitute the source of the syncytial trophoblast throughout the period of growth of the villi.

It is suggested that the products of the uterine glands are probably a more important source of nutrients to the developing conceptus in this species than in others with a villous haemochorial placenta.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Early implantation. Although the ectodermal plate is elongate, there is as yet no separation into separate embryos. The yolk-sac (uppermost membrane in picture) is already inverted. The amnion has been formed but is not yet distended with fluid. The exocoelom separates the amnion from the trophoblast in the central region. Note the masses of trophoblast at the margin of the implantation site. Tetrachrome stain. × 110.

Fig. 2. Implantation site from the avillous stage. Parts of three of the four embryos are visible in the picture. The fundic tip of the uterus was everted prior to fixation, and the blood has been washed out of the maternal blood sinuses at the base of the endometrium. Note the extensive central trophoblast. H. and E. × 28.

Fig. 3. Marginal region of the implantation site from the section in fig. 2. At the top of the picture is the inverted yolk-sac placenta. It fuses with the trophoblast in the middle of the picture. The trophoblast extends into the endometrium at the bottom of the picture. Note that the

luminal epithelium of the endometrium is folded where the trophoblast penetrates the endometrial tissues. H. and E. × 110.

Fig. 4. Margin of an implantation site from early in the period of expansion of the placenta. At the top of the picture the well-vascularized inverted yolk-sac lies in the uterine lumen. Note that the cell columns and vascular villi have extended into the endometrial sinuses in the centre and left of the picture. Tetrachrome stain. × 43.

Fig. 5. A junctional zone from the same stage as fig. 4. In the upper left of the picture is the inverted yolk-sac epithelium. In the upper right is syncytial trophoblast. Note the tongue of trophoblast extending into the endometrium in the lower left corner and the luminal epithelial cells in the centre of the picture. Tetrachrome stain. × 200.

Fig. 6. A portion of the placenta from late pregnancy. The maternal sinuses have been greatly expanded. The endometrium consists of thin arcades over the sinuses and is fused with the overlying placental membranes. The endometrial blood vessels are seen in the radiating septa. Note the dilation of the luminal portion of such a vessel in the upper centre of the picture. H. and E. × 11.

PLATE 2

Fig. 7. Section from the same specimen as Pl. 1, fig. 2. The two embryos seen in the upper right of the picture are as yet incompletely separated. A portion of the yolk-sac placenta appears in the upper left corner. Note the masses of cells occurring in the lower centre and right of the picture. These masses constitute the earliest stage in the formation of cell columns. H. and E. × 110.

Fig. 8. Development of the cell columns is more advanced than seen in fig. 7. The cell columns in the lower portion of the figure are more highly developed than those in the upper portion of the picture. In the right centre, a vascular villous is seen in the early stages of formation. H. and E. × 110.

Fig. 9. Typical cross-section of the cell columns and vascular villi during the period of expansion of the placenta. Note the uterine glands at the margins of the picture. H. and E. × 110.

Fig. 10. Villi from the period of the mature placenta. Note the relative density of the mesodermal core and the peripheral position of the capillaries. There is a fibrotic area in the endometrium overlying these villi. H. and E. × 140.

Fig. 11. A formalin-fixed, unstained, terminal frond, dissected from a villous stem early in the period of placental expansion. The light margin of the projection is formed by the cell columns. At the upper right an anastomosis with another frond was broken. A particularly flat frond was selected for photography. × 43.

Fig. 12. A portion of a villous stem from a mature placenta. The treatment and magnification are the same as in fig. 11. Note that the terminal projects are relatively uniform in diameter although knobby. Several anastomotic branches between this and other terminal arborizations had to be broken during the dissection. The vascular pattern is particularly clear in this specimen. × 43.

PLATE 3

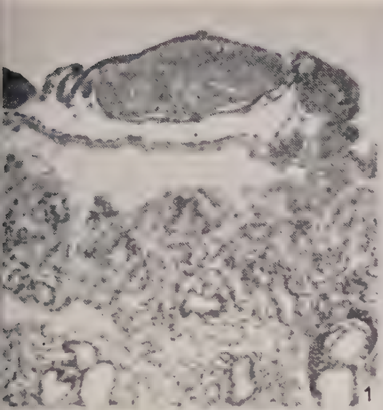
Fig. 13. Period of placental expansion. A cell column is seen in the centre of the picture. To the lower right and left are endometrial glands. Note that the endothelium of the maternal sinus is still present, and that the syncytium surrounding the cell column is continuous with that of the vascular villi above it. Tetrachrome stain. × 435.

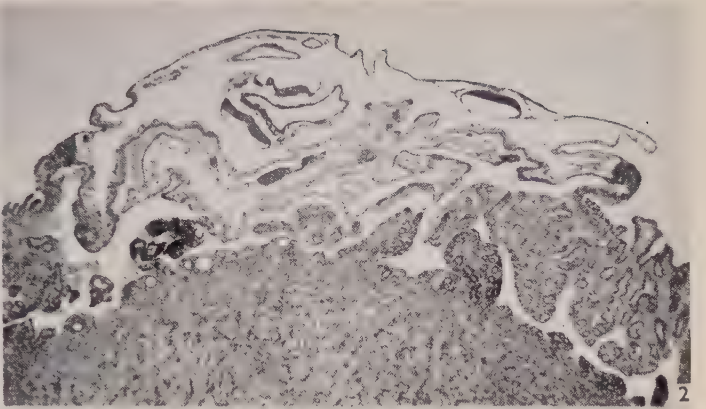
Fig. 14. A portion of a villus from a mature placenta. Note the Golgi zone underlying the nucleus of the fibroblast in the lower centre of the picture, and that the fibroblasts in general are glandular in appearance. The areas of cytoplasm which appear light in this picture exhibit basophilia in other types of preparation. Note also the irregularity of the surface of the syncytium. Champy's fixed, post-osmified, phase contrast. × 790.

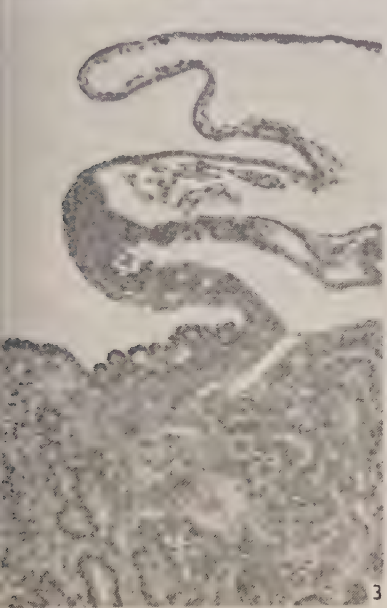
Fig. 15. A tangential section of the syncytium illustrating the presence of numerous small vacuoles and the irregularity of the free surface. Osmichrome fixation, PAS, haematoxylin. × 435.

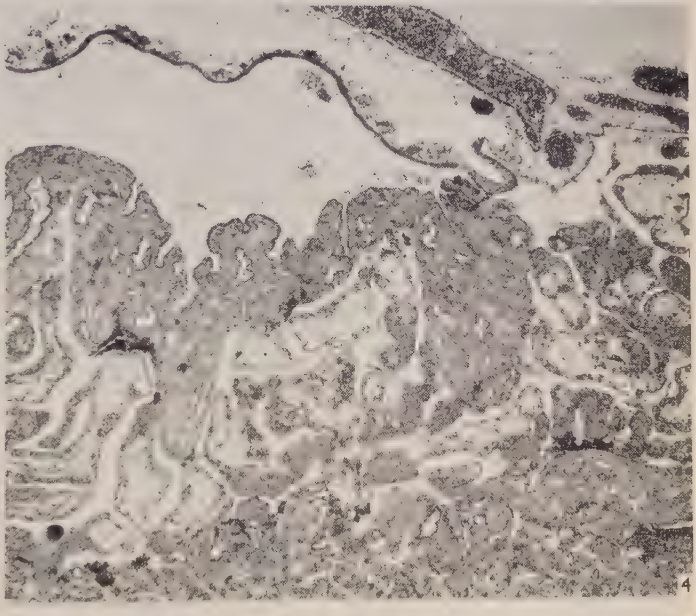
Fig. 16. Portions of two villi. Note the presence of a large vacuole in the syncytium at the centre of the picture and the variable thickness of the basement membrane. Period of placental expansion. Osmichrome fixation, PAS, haematoxylin. × 1000.

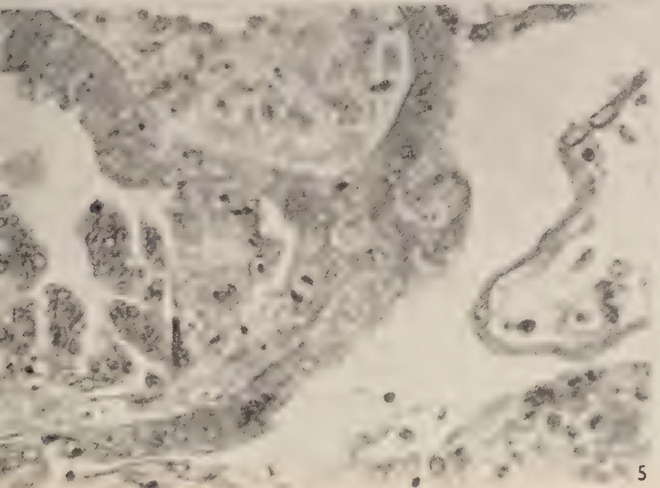
Fig. 17. Syncytium of vascular villi from expansion period. Note the presence of a brush border and of protoplasmic projections. Phase contrast, osmium tetroxide fixed, methacrylate embedded section. × 790.

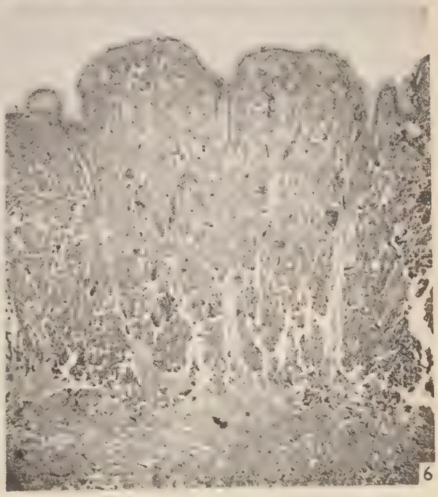












ENDERS—DEVELOPMENT AND STRUCTURE OF THE VILLOUS HAEMOCHORIAL PLACENTA OF THE NINE-BANDED ARMADILLO (*DASYPUS NOVEMCINCTUS*)

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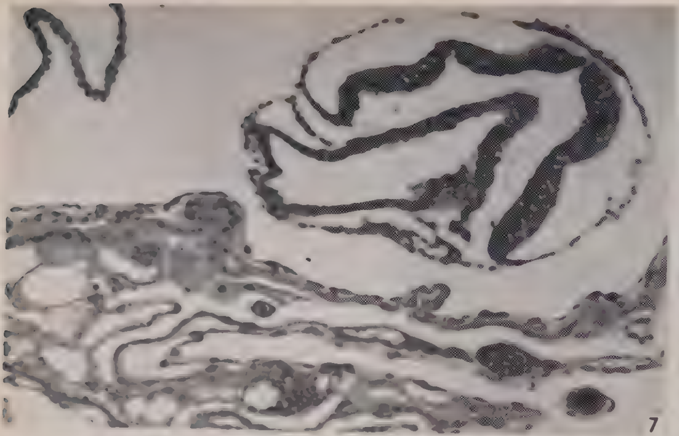

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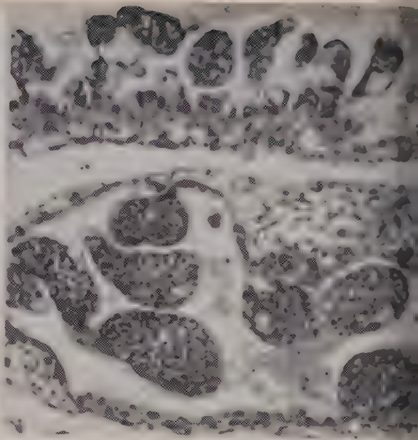

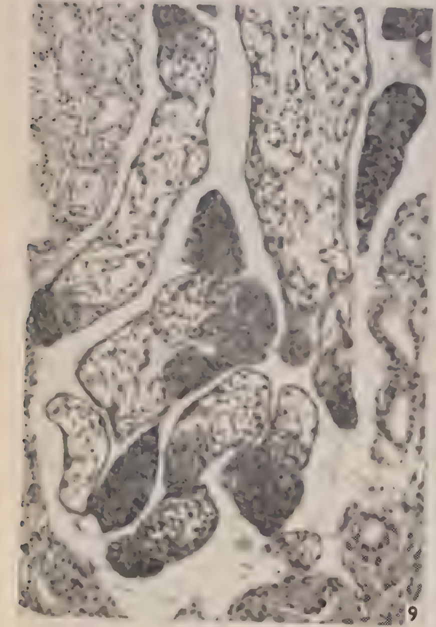

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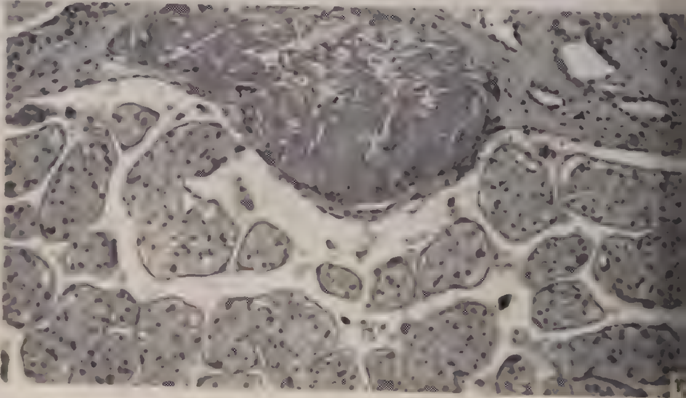

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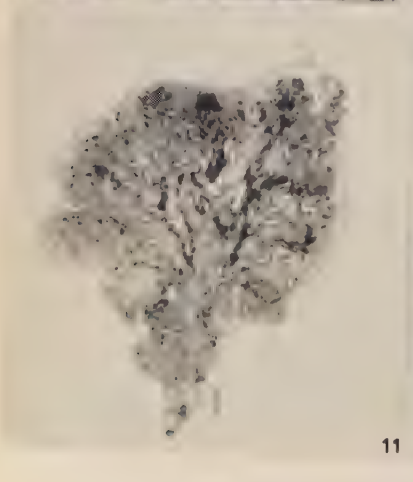

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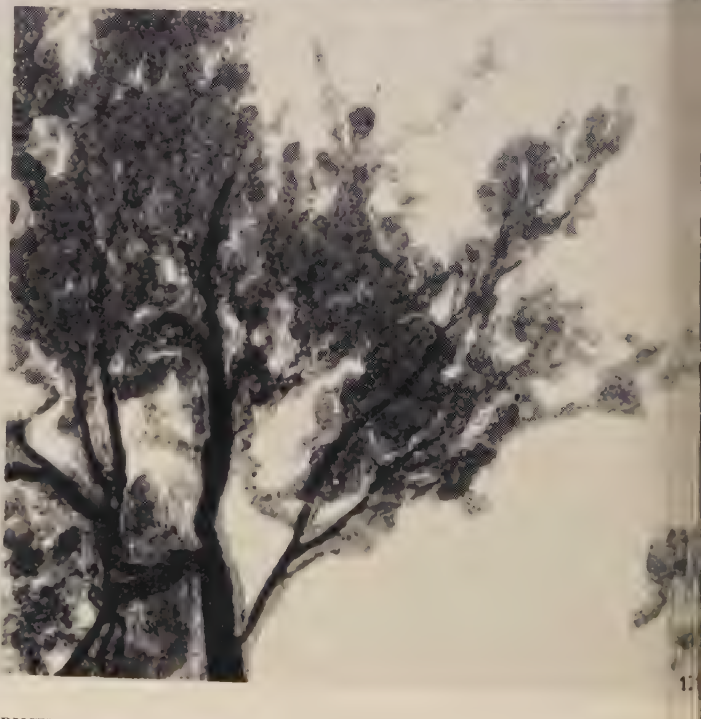

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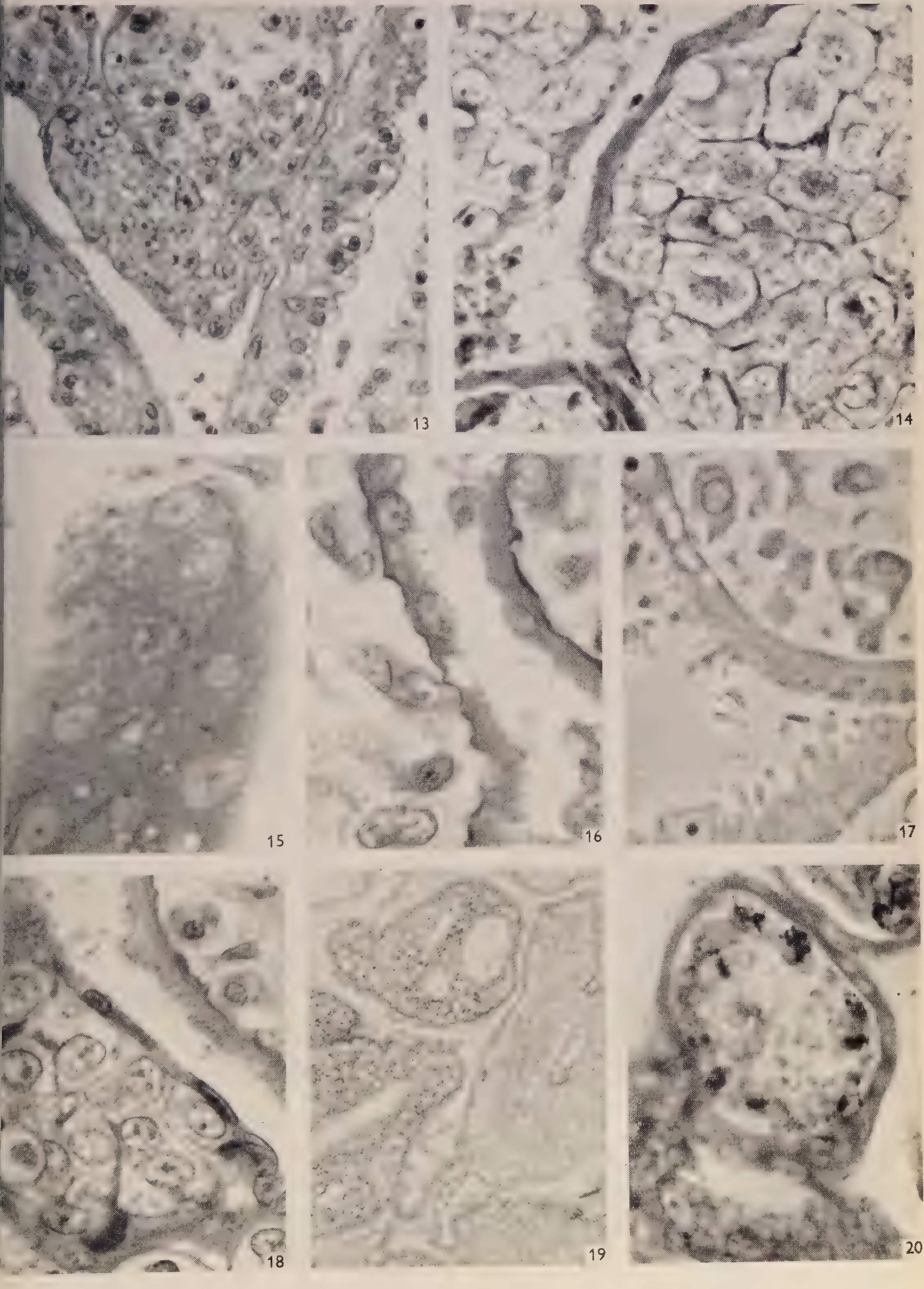
ENDERS—DEVELOPMENT AND STRUCTURE OF THE VILLOUS HAEMOCHORIAL PLACENTA OF THE NINE-BANDED ARMADILLO (*DASYPUS NOVEMCINCTUS*)



ENDERS—Development and structure of the villous haemochorial placenta of the nine-banded armadillo (*Dasypus novemcinctus*)

Fig. 18. Cell column. Note the syncytium surrounding the cell column and the presence of a dark substance between the cells within the column. This intercellular substance is PAS positive in other types of preparation. Osmichrome fixation, iron haematoxylin, aniline blue. × 1000.

Fig. 19. Glycogen in the vascular villi. Note that there is appreciably more glycogen in the villi containing the larger blood vessels. Carnoy's fixed, PAS. × 110.

Fig. 20. Frozen section demonstrating lipid in a cell column and vascular villus. Note that the lipid droplets in the cell column are small and that there are numerous large droplets associated with the blood vessels of the vascular villus. Formalin fixation, Sudan Black B. × 435.

OBSERVATIONS ON CARBOHYDRATE MATERIALS IN THE RAT PLACENTA

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There is a considerable body of previous work, both histological and histochemical, on the rat placenta. The histochemical descriptions are, however, in some respects incomplete and much of the work was carried out with techniques which are not now considered entirely satisfactory. Since the histochemistry of the placenta is clearly relevant to many problems of placental function, it was felt that the previous findings should be checked, and, where possible, amplified. The present paper describes a series of studies on the carbohydrate material of the rat placenta between 10 and 17 days, when most of the elements of the placenta become fully formed. It is hoped to amplify this information in later publications.

MATERIALS AND METHODS

Rat placentae (hooded Rowett strain) of between 10 and 17 days were submitted to fixation in 10 % formalin, Bouin's fluid, Rossman's fluid, Carnoy's fluid, acetic alcohol formalin (Lillie, 1954), acetone, or by the method of Lison & Vokaer (1949) in Gendre's fluid. In order to compare the efficacy of different fixatives for the preservation of carbohydrate material a series of 12-day placentae were submitted to the following procedure. Each placenta was bisected. One half was fixed in 10 % formalin and the other in Carnoy's fluid, Rossman's fluid, Bouin's fluid or acetic alcohol formalin. This procedure was carried out both at room temperature and, with the duration of fixation doubled, at 4° C. With another specimen, half was fixed in 10 % formalin at room temperature and half in 10 % formalin at 4° C. for twice as long. With yet another specimen, half was fixed in 10 % formalin at room temperature and the other half in Gendre's fluid at $-73°$ C. by the method of Lison and Vokaer.

All the specimens were embedded in paraffin and sectioned throughout the entire placental region. With some the complete series was mounted. With others every twenty-fifth or every fiftieth section was mounted throughout the whole placenta to make a set of slides for staining by the periodic acid-Schiff technique, and intermediate sections were mounted to make at least four similar sets for staining by other methods. Each specimen was, therefore, investigated throughout the whole placenta and the findings are not dependent upon a few isolated sections.

Carbohydrates were investigated by the PAS technique. Glycogen was identified by digestion for one hour at room temperature in a 1 % solution of diastase in phosphate buffer at pH 6·5. Diastase digestion was always carried out on a section immediately adjacent to one stained by the PAS technique without digestion. In addition, glycogen was demonstrated by using a short dimedone blockade in combination with the PAS technique (Bulmer, 1959). This too was controlled by diastase digestion.

Glycoproteins, mucoproteins and mucopolysaccharides were further studied by staining in alcian blue, used as a 1 % aqueous solution (Steedman, 1950) or as a 1 % solution in $\frac{1}{2}$ % acetic acid in the method of Lison (1954). Some sections were stained in azur A (Kramer & Windrum, 1955). This was also carried out on sections which had been sulphated in an equivolume mixture of sulphuric acid and glacial acetic acid at 4° C. (Moore & Schoenberg, 1957). The methylene blue extinction points of some of the carbohydrate materials were ascertained by the method described by Pearse (1953), and the presence of a protein component in many of the PAS-positive materials was confirmed by the coupled tetrazonium reaction (Danielli, 1953), using tetrazotized *o*-dianisidine and *β*-naphthol. In addition, attempts were made to stain carbohydrate materials by Gomori's aldehyde fuchsin technique (Pearse, 1953), without prior oxidation, and by the method for mucoproteins described by Leach (1947).

Occasional sections were stained for reticulin fibres by the method of Long (1948) and for alkaline phosphatase activity by the calcium–cobalt and azo-dye methods described by Gomori (1952).

The general topography of the placenta was studied in sections stained by a trichrome technique, using *ponceau de xylidene* as the plasma stain and aniline blue as the fibre stain.

OBSERVATIONS

It may first be advisable to refer briefly to the morphology of the rat placenta during the 10–17 day period, as we have found it in our trichrome preparations. Fuller and more detailed accounts may be found in the works of Duval (1891) and Bridgman (1948).

At 10 days the mesometrial decidua, which forms the maternal side of the allantoic placenta, is well developed, and may roughly be divided into three main zones. The outer zone lies immediately internal to a layer of unchanged stroma just beneath the uterine muscle. Histologically it resembles the unchanged stroma itself, except for a large content of the granular cells of the metrial gland (Selye & McKeown, 1935) principally distributed around the blood vessels. Beneath this, the middle zone consists of large cells with an evenly staining, rather basophil cytoplasm. Some granulated metrial-gland cells also occur in this zone. The inner zone, which abuts against the foetal tissues, contains large areas of cells with completely unstained cytoplasm. Between these areas are cells with evenly staining cytoplasm, resembling those of the middle zone.

The antimesometrial decidua is a fairly thick layer and the new uterine lumen is present on each side, though it does not extend to the antimesometrial pole. The antimesometrial decidua is surrounded by a yellow or yellowish brown-staining capsule, which appears to correspond to the 'fibrous capsule' described by Bridgman (1948). It extends round as far as the junction with the mesometrial decidua and contains many small nuclei. The antimesometrial decidua itself contains large cells with a moderately basophil cytoplasm, rather resembling the cells of the middle zone of the mesometrial decidua. Between these cells is a fairly thick network of yellow-staining material, which resembles that of the 'capsule' and which also contains many small nuclei.

At the 12-day stage the antimesometrial decidua is much thinner and forms the decidua capsularis. It consists of a mass of 'symplasma' (Bridgman, 1948) in which nuclei of varying shapes and sizes are distributed. It is surrounded by the yellowish-staining 'capsular' layer, which extends for a short distance into the mesometrial decidua between the peripheral parts of the middle and outer zones. The uterine cavity is continuous around the antimesometrial pole. The arrangement of the foetal portion of the placenta is shown in Pl. 1, fig. 1.

By the 17-day stage Reichert's membrane has ruptured and recoiled to the margin of the allantoic placenta, where a small remnant of the decidua capsularis, now devoid of nuclei, is still attached. The 'capsular' layer starts at the periphery of the allantoic placenta from this remnant of decidua capsularis and extends mesometrially around the outside of the placenta for some distance, disappearing before it reaches the mesometrial pole. Here, in the region where the 'capsular' layer is deficient, a mass of tissue projects from the placenta, forming a bulge into the mesometrium and stretching the uterine muscle, which is an attenuated and often interrupted layer. This mass of tissue consists mainly of two types of cell—granulated metrial gland cells and large cells with completely unstained cytoplasm. Areas of cells similar to those of the latter type occur also amongst the cytotrophoblast of the junctional zone. It is often difficult to distinguish between the clear-staining cells of the decidua and those of the junctional zone.

Histochemical findings

The results of the differing methods of fixation on the members of the series of 12-day placentae were first investigated. No obvious differences could be detected in the amounts of carbohydrate demonstrable after the various fixatives. Each method of fixation preserved glycogen and diastase-fast PAS-positive materials in the same tissues and the intensity of the PAS reaction was approximately constant. There was, as might be expected, a varying degree of glycogen 'flow' with differing fixatives. It would seem that all the fixatives which we used are equally satisfactory for the preservation of PAS-positive materials in the rat placenta. It is interesting to note that Davies (1956) considered placental glycogen in the rabbit to be extremely stable to histological fixation.

The distribution of the various PAS-positive materials in the different parts of the placenta is described below.

Decidua

In the 10–14-day placentae there are large amounts of glycogen in the inner zone of the mesometrial decidua, occupying the cytoplasm of the large clear-staining cells which were identified in the trichrome preparations. The aggregations of these cells constitute the 'glycogen areas' of the decidua (Pl. 1, figs. 2, 3). The individual cells of the glycogen areas are separated from one another by intercellular material which gives a strongly positive diastase-fast PAS reaction (Pl. 1, fig. 3). The middle and outer zones of the mesometrial decidua contain only small amounts of glycogen, mainly in the cytoplasm of the granulated cells of the metrial gland. These latter cells Wislocki, Weiss, Burgos & Ellis (1957) found to be characterized by the strong diastase-fast PAS reaction of their acidophil granules. It is, of course, difficult to make certain that all the acidophil granules are PAS-positive. However, in a section which has been submitted to the PAS technique it is not possible, by using acidic stains, to demonstrate any granules which are not already coloured by Schiff's reagent. The acidophilia of the granules is not destroyed by the oxidation, as one might suspect from the work of Dempsey, Singer & Wislocki (1950), since it can be demonstrated in preparations which have been exposed to periodic acid. The cytoplasm of the basophil cells of the middle zone gives a diffuse diastase-fast PAS reaction. There is little glycogen in the antimesometrial decidua, though small patches do occur. The basophil cells of the antimesometrial decidua of the 10-day placenta give a similar PAS reaction to that of the basophil cells of the middle zone of the mesometrial decidua, and the capsular material, yellowish-staining in the trichrome preparations, is also PAS-positive and diastase-fast. This reaction, however, is rather patchily distributed and more intense in some regions than in others.

In the 17-day placenta both types of cell in the mesometrial decidua contain glycogen. The metrial gland cells, as in the earlier stages, contain glycogen and diastase-fast granules. The other cells contain glycogen (Pl. 1, fig. 4) and are separated from each other by diastase-fast intercellular material. The remnant of decidua capsularis, which contains no glycogen, gives, like the capsular layer around the periphery of the allantoic placenta, a diastase-fast reaction.

In all our specimens the uterine contents and the distal borders of the uterine epithelial cells give diastase-fast PAS reactions. In neither of these situations were we able to demonstrate glycogen.

A feature of considerable interest, though doubtful significance, is the occurrence of a positive reaction with the dimedone-PAS technique in some of the maternal vessels of 12–17-day placentae. This is diffusely distributed, and is detected in every section in two or three of the vessels of the inner layer of the mesometrial decidua. It has not been observed in sections which have been pre-digested with diastase. In ordinary PAS preparations it is difficult to be certain of the presence of diastase-soluble material in the maternal vessels, because of the diastase-fast reaction of the fibrin. Nor do preparations stained with Best's carmine give a convincingly positive reaction in the vessels. It is, however, possible that a substance giving some of the histochemical reactions of glycogen occurs in the maternal vessels. Wolman & Feingold (1953) pointed out that diastase-soluble materials other than glycogen may be PAS-positive. Glycogen in the maternal vessels was described by Goldmann (1912), while Bridgman found considerable numbers of metrial gland cells lying free in the maternal vessels. She suggested that these might be a possible source of intravascular glycogen. In our material, while we have seen them, metrial gland cells in this position have been uncommon.

Giant cells

The distribution of glycogen in the giant cells is patchy, and in any one section glycogen is demonstrated in only a very small proportion of the giant cells (Pl. 1, fig. 5). This proportion is usually higher in the giant cells which lie against the inner zone of the mesometrial decidua than in those which lie peripherally, around the outside of Reichert's membrane. At the 17-day stage the glycogen content of the giant cells appears to be reduced.

A striking feature of the giant cells is the presence of cytoplasmic granules of diastase-fast PAS-positive material. These vary in size from very fine granules to large globules of the size of a red blood cell (Pl. 1, fig. 6). They occur in every stage which we have examined, but appear to be less numerous at 17 days than at 10–14 days.

Trophoblast

There is an abundance of glycogen in the 'glycogen cells' of the spongy or junctional zone trophoblast. At the 10–14-day stage the cells of the junctional zone have a deeply basophil cytoplasm. The glycogen cells occur in clumps, usually separated from the maternal vessels by other cytotrophoblast cells, and only a small proportion of them have cytoplasmic vacuoles. Most of their glycogen is diffusely distributed throughout the basophil cytoplasm, and this is particularly evident in the specimens fixed by the method of Lison and Vokaer (Pl. 2, fig. 7). In the 17-day placenta the glycogen areas of the junctional zone consist of cells with a cytoplasm which no longer takes the basic stain in a trichrome preparation, but which contains large granules of glycogen (Pl. 2, fig. 8). Individually, as Bridgman pointed out, these cells are indistinguishable at this period from the glycogen cells of the decidua.

Diastase-fast intercellular material separates the cells of the junctional zone at 10–14 days, but tends to be rather indistinct. At the 17-day stage it is extremely well marked, and clearly indicates that the junctional zone trophoblast is not a syncytium. Most cell areas contain only one nucleus, but some are binucleate. Around the larger maternal vessels of the junctional zone the PAS reaction of the intercellular material is particularly intense. Intercellular material can be identified between the glycogen cells, but only discontinuous segments of this give a diastase-fast PAS reaction.

In the 10-day placenta the laminae, and in the 12-day placenta the labryrinth to which they give rise, contain no PAS-positive material. At 14 days fine granules of glycogen are distributed throughout the labyrinth (Pl. 2, fig. 9). At 17 days the labyrinth contains rather less glycogen than at 14 days, and there is a roughly equal distribution of fine granules of PAS-positive diastase-fast material. The boundaries of the maternal blood spaces of the trophoblast give a rather faint diastase-fast PAS reaction, and this is more marked at the 14-day stage than at either 12 or 17 days (Pl. 2, fig. 10).

Yolk sac

The visceral layer of the yolk sac epithelium contains no glycogen until 14 days, when it can be demonstrated, usually basally, in some of the yolk sac cells. At the 17-day stage large quantities of glycogen occur in most cells, distributed throughout the cytoplasm (Pl. 2, fig. 11). Diastase-fast PAS-positive material is found in the visceral yolk sac epithelium in all our specimens, towards the distal poles of the cells (Pl. 2, fig. 12). Supranuclear granules of varying size, but which seem to be larger and more numerous at 14 days than in the earlier or later stages, can be distinguished from the sharply stained distal border of the cell, which Wislocki & Dempsey (1955), by electron microscopy, confirmed to be a brush border. Though our findings, except for our demonstration of the absence of glycogen before the 14-day stage, largely agree with those of Wislocki & Padykula (1953), our material does not always confirm their observation of a clear zone between the PAS-positive brush border and the PAS-positive supranuclear granules. This can occasionally be seen in specimens fixed in alcoholic fluids, but does not occur after fixation in formalin or by the method of Lison and Vokaer (Pl. 2, fig. 12). It is most obvious in a specimen fixed in ice-cold acetone. Nor do we find any appreciable differences in the intensity of the PAS reaction of the supranuclear granules at the various stages we have examined. Our findings on the reactions of Reichert's membrane, the parietal layer of the yolk sac epithelium, the 'visceral' and 'serosal' basement membranes and the vitelline pockets of the chorio-allantoic placenta confirm those of Wislocki & Padykula.

The endovascular plasmodium

The thickening of the lining of many of the vessels of the mesometrial decidua is apparent in the 10-day placenta. The cells are rather small, with a basophil cytoplasm. In the 12-day placenta the lining cells are large, and of similar size to those of the junctional zone trophoblast. Their intensely basophil cytoplasm makes them resemble trophoblast cells, and they appear to be in continuity with the junctional zone trophoblast. Only rarely is it possible to demonstrate glycogen in any of these cells, but one does see granulated metrial gland cells, containing glycogen, in close association with them. A striking feature is the presence of a basement membrane around the outside of the endovascular plasmodium, separating it from the decidua. This membrane, which is sometimes incomplete, gives an intense diastase-fast PAS reaction (Pl. 3, fig. 13), and is also stained by Long's method. Cell boundaries can be detected in the endovascular plasmodium, but they are only faintly PAS positive. At the 17-day stage the endovascular plasmodium retains its intense cytoplasmic basophilia, and the basement membrane which surrounds it is now complete. Again, glycogen is only rarely found in any of these cells, but the metrial gland cells and glycogen cells of the decidua are closely related to them.

Further investigations of diastase-fast PAS-positive materials

Since none of the diastase-fast PAS-positive materials which we demonstrated is sudanophilic, and with the coupled tetrazonium technique all give positive reactions (Pl. 3, fig. 14) which are abolished or greatly reduced by benzoylation, it is likely that they all consist of mucoprotein or glycoprotein. However, none stained significantly with Leach's Bismarck brown technique, and the only sites to give appreciable staining with aldehyde fuchsin were Reichert's membrane, the distal brush borders of the visceral yolk sac cells and the capsular layer of the decidua. With alcian blue, staining occurred only in the distal brush border of the visceral yolk sac epithelium, and even this was marked only in specimens which had been fixed in formalin. This may presumably be associated with the increased tissue acidity produced by formalin fixation (Dempsey & Wislocki, 1946).

Staining with azur A produced alcohol-fast metachromasia in some of the supranuclear granules of the visceral yolk sac cells. We were unable to demonstrate any alcohol-fast metachromasia in the metrial gland cell granules. With aqueous azur A, without dehydration, there was metachromatic staining in the intercellular material of the decidua, and, fairly faintly, in the metrial gland cell granules. The diastase-fast inclusions in the giant cells were not metachromatic, staining a greenish colour similar to that shown by fibrin and red blood cells.

After sulphation, staining in azur A produced a very different picture. With the exception of fibrin in the maternal vessels all the PAS-positive materials exhibited metachromatic basophilia. The staining of the diastase-fast materials was seen more clearly in sections which had been digested with diastase, since the glycogen appeared to be diffused by sulphation and tended to overlie the other materials. The diastase-fast granules and globules in the giant cells were extremely well demonstrated after sulphation, and many of the giant cells were seen to have a ring of fine granules immediately around the nucleus (Pl. 3, fig. 15). These perinuclear granules are only very faintly PAS-positive. Prolonged digestion with diastase or ribonuclease before sulphation does not alter the metachromatic basophilia induced in these structures in the giant cell cytoplasm, and the staining is therefore unlikely to be due to cytoplasmic nucleic acid. Moreover, the pattern of staining in the giant cell cytoplasm after sulphation, with the granules and globules of varying sizes, is quite different from the rather reticular pattern, due to ribonucleic acid, seen in a section stained in azur A without previous sulphation.

The boundary line between the maternal blood spaces and the trophoblast in contact with them is very well marked after sulphation (Pl. 3, fig. 16), and it is interesting that this boundary line also contains alkaline phosphatase. The distribution of alkaline phosphatase in this site has been described by Wislocki, Deane & Dempsey (1946), Pritchard (1947) and Padykula (1958), all of them using calcium–cobalt techniques. In our azo-dye preparations we have obtained a very precise linear distribution of alkaline phosphatase activity (Pl. 3, fig. 17). Borghese (1957) believed that activity in this site, between the maternal blood and foetal tissues, may be associated with the transfer of materials across the placental barrier.

An interesting feature is the failure of sulphation to induce metachromatic staining in the fibrin of the blood vessels. An attempt to produce metachromatic basophilia in the PAS-positive materials by prolonged chromic acid oxidation (Lison, 1953; Burkl, 1953) was unsatisfactory and successful only with the glycogen.

An attempt was made to differentiate between the diastase-fast PAS-positive materials in the various parts of the placenta by comparing their methylene blue extinction points. All these tests were carried out on formalin-fixed material, and the pH values of the methylene blue solutions were checked with a pH meter. Staining of the diastase-fast granules and globules of the giant cell cytoplasm, the red cells, the fibrin of the maternal vessels, Reichert's membrane and the contents of the uterine and yolk sac cavities became extinct between pH 6·6 and pH 5·6. At pH 5·6 the distal borders of the visceral yolk sac cells and most of their supranuclear granules continued to stain, though there was some variation from cell to cell. In addition, most of the diastase-fast granules of the metrial gland cells were still stained. The intercellular material between the glycogen cells of the decidua was strongly stained. At pH 4·8 a small proportion of metrial gland cell granules, the distal borders of some of the yolk sac cells and occasional supranuclear granules continued to stain. The intercellular material between the glycogen cells was faintly stained. At pH 3·75 the only PAS-positive structure to stain with methylene blue was the capsular layer which surrounds the decidua capsularis and extends mesometrially between the middle and outer layers of the mesometrial decidua (Pl. 3, fig. 18).

DISCUSSION

While there have been many histochemical investigations of the rat placenta, there has been no comprehensive study of the PAS reactions throughout the whole placenta. The only publications describing the results of the PAS technique have been those of Wislocki & Padykula (1953) on the yolk sac and Wislocki, Weiss, Burgos & Ellis (1957) on the granular cells of the metrial gland. Previous workers have described the distribution of glycogen in the placenta, using either Best's carmine (e.g. Bridgman, 1948) or the Bauer-Feulgen technique (Wislocki, Deane & Dempsey, 1946). Only Wislocki & Padykula and Wislocki, Weiss, Burgos & Ellis make it clear that digestion controls were used.

Our findings on the distribution of glycogen in the metrial gland cells and in the yolk sac cells essentially confirm those of Bridgman; Wislocki, Weiss, Burgos & Ellis and Wislocki & Padykula. We show that there is no glycogen in the visceral yolk sac epithelium at 12 days—a stage at which Wislocki & Padykula did not use digestion controls. We confirm the presence of fine flecks of glycogen in the labyrinthine syncytium at 14–17 days, as Goldmann (1912) and Bridgman described. Wislocki, Deane & Dempsey were unable to demonstrate glycogen in this situation with the Bauer-Feulgen technique.

Our account of the glycogen cells of the junctional zone trophoblast corresponds essentially with that of Bridgman. There has been considerable controversy about the origin of these cells since their first description by Duval (1891), who considered them to be of maternal origin. This view has been corroborated by recent workers, and Pritchard (1947) considered them to be 'morphologically and histochemically identical' with metrial gland cells. On the other hand, Jenkinson (1902) described the foetal origin of the trophoblastic glycogen cells in the mouse. He found that the trophoblast cells, as they came to acquire their glycogen, became increasingly vacuolated. There seems no evidence to suggest that the junctional zone glycogen cells of the rat have a maternal origin. We are unable to agree with Pritchard's view that they are identical with metrial gland cells. While many of the trophoblastic glycogen cells and many of the metrial gland cells are binucleate, the presence of the acidophil and diastase-fast PAS-positive granules in the metrial gland cells and the intense cytoplasmic basophilia of the glycogen cells of the junctional zone in the earlier stages, which in trichrome preparations often makes them indistinguishable from other trophoblast cells of this region, demonstrates that there are considerable differences between the two types of cells. Their common absence of alkaline phosphatase activity, cited by Pritchard, does not distinguish the glycogen cells from other trophoblast cells of the junctional zone or the metrial gland cells from other decidual cells (Padykula, 1958). There seems, therefore, no reason to suggest that the glycogen cells of the junctional zone are anything other than derivatives of the cytotrophoblast. We find no evidence to support the view of Szendi (1933) that the glycogen areas of the trophoblast are lymphatic spaces containing glycogen cells which are migrating from the maternal side of the placenta to the foetal side.

The possibility of the existence of glycogen in the maternal vessels is of considerable interest, since Boyd (1957) has described glycogen in the maternal and foetal vessels of the human placenta. Like him, we are not satisfied that the intravascular diastase-soluble material is glycogen, or that it is not a fixation artefact. Using Best's carmine, Goldmann described glycogen in the maternal vessels of the rat placenta. He also pointed out the similarity between the distribution of glycogen particles in the syncytium and the distribution of vitally ingested particles of Trypan blue. Bridgman, referring to Goldmann's views, extended them by indicating the similarity between the patchy distribution of glycogen in the giant cells and the patchy distribution of vitally ingested Trypan blue. She suggested that the glycogen of the giant cells may actually be ingested as glycogen.

The significance of the placental glycogen has been the subject of discussion since Bernard (1859) suggested that it constitutes a part of the glycogen store for the embryo during the period before its liver assumes that function. After the lapse of a hundred years it is still impossible to do more than speculate. In the rat placenta the principal sites of glycogen deposition are the inner zone of the decidua and the junctional zone trophoblast. Selye & McKeown (1935) suggested that glycogen is transferred as such from the decidua to the foetal tissues by the passage of metrial gland cells along the maternal vessels. Bridgman adduced evidence in support of this view. Apart from any other consideration, the infrequency with which we have found metrial gland cells in this situation makes us doubt whether this is a significant mechanism.

Few of the PAS-positive diastase-fast materials in the rat placenta have been reported previously. The granules of the metrial gland cells were investigated histochemically by Wislocki, Weiss, Burgos and Ellis, who suggested that they may be the source of the relaxin which is known to be formed in the rat placenta (Zarrow, 1956). We have obtained a methylene blue extinction point for these granules lower than that described by Wislocki and his colleagues, but this may be explained by the use of different fixatives. The metachromasia demonstrated by Wislocki *et al.* (1957) and by Asplund, Borell & Holmgren (1940), after lead acetate fixation, was poorly marked in our specimens, and was removed by dehydration in alcohol. It is clear that the granules consist of glycoprotein material, and because of their close association with the maternal vessels it is tempting to suggest that they represent a site of secretion of placental hormone. The evidence that they are relaxin, however, is inconclusive, particularly since relaxin appears to be a simple protein (Frieden & Hisaw, 1953).

The PAS-positive brush border and the PAS reaction of the supranuclear granules of the visceral yolk sac epithelium were described by Wislocki and Padykula, who suggested that they might indicate absorption from the PAS-positive contents of the yolk sac cavity and the uterine lumen. The experiments of Gerard (1925) on the mouse and the rabbit indicate that such absorption may occur, and this evidence seems more significant than a common PAS reaction of the various materials concerned. Moreover, these materials do not appear to be identical. The distal borders of the yolk sac cells and the supranuclear granules show a lower methylene blue extinction point than the yolk sac or uterine contents, and the distal borders are the only placental structures to stain significantly with alcian blue.

The PAS-positive inclusions in the giant cells have not been reported previously. They are more numerous at 10 days than at 17 days, but the significance of their origin and decrease is at present completely obscure. Many workers have ascribed phagocytic properties to the giant cells. For example, Bridgman stated that at the ninth day they appear to contain ingested red cells. In our trichrome preparations the larger PAS-positive globules very much resemble red cells, and it is possible that they have been so interpreted by earlier workers. In the mouse, however, Jenkinson described granular inclusions in the giant cells, and his illustration of these suggests that they correspond with the diastase-fast PAS-positive granules which we describe in the rat.

The PAS-positive intercellular substance is present in large amounts in both foetal and maternal tissues. Davies (1956), working on the rabbit, suggested that the intercellular material of the decidua constitutes a barrier to invasion by the foetal tissues. While this might be correct, little is known about the mechanics of invasiveness of foetal tissues. The reactions of the 'capsule', which encloses the decidua capsularis and extends into the mesometrial decidua, are interesting. Its yellowish stain in the trichrome preparations, with its low methylene blue extinction point, suggest that Bridgman's description of it as a fibrous capsule may be incorrect. Amoroso (1952) mentions the presence of muscle fibres in the decidua capsularis. We find no evidence of this; nor would it be expected if Sabotta's description (1903) of the origin of the new uterine lumen from the remaining basal segments of the uterine glands is correct. The intercellular material of the foetal tissues demonstrates the persistence of cell boundaries in the junctional zone at 17 days. Duval described the complete syncytialization of all the cytotrophoblast, but Bridgman pointed out that if the glycogen cells of the junctional zone were of trophoblastic origin this was clearly incorrect. Moreover she found that cell boundaries did persist to some extent in the rest of the junctional zone trophoblast. Some of the 'intercellular walls' at 17 days are quite thick and stain a pale blue colour in our trichrome preparations. Sometimes binucleate cells are seen, but usually the PAS-positive intercellular material separates cells with single nuclei.

The endovascular plasmodium of the rat placenta, first described as such by Duval, has been a further feature of controversy. Duval considered that the large cells lining many of the maternal vessels of the decidua were derivatives of the cytotrophoblast, and Bridgman supported this view. Jenkinson denied the existence of an endovascular plasmodium in the mouse. The intense cytoplasmic basophilia of the endovascular plasmodium of the rat placenta and its frequent apparent continuity with the junctional zone trophoblast suggest the conclusion that it is derived from the trophoblast. More evidence is needed, however, before this can be regarded as proven. The PAS-positive basement membrane which separates the endovascular plasmodium from the surrounding decidua is an interesting feature. Cell boundaries can be detected between the cells of the endovascular plasmodium but these are only very faintly PAS-positive. We find no evidence that the endovascular plasmodium is a syncytium, and a similar view was expressed by Orsini (1954) for the hamster.

Our findings have largely confirmed those of Bridgman (1948), Wislocki & Padykula (1953), and Wislocki *et al.* (1957). In addition, we report the occurrence of many PAS-positive and diastase-fast materials in the rat placenta, but we feel that speculation on the significance of many of these is both premature and inadvisable. The solution of such problems requires the correlation of histochemical findings with those of physiology and biochemistry. Otherwise, hypotheses concerning the significance of the occurrence and distribution of the various materials are based on inadequate evidence.

SUMMARY

1. The carbohydrate material of the rat placenta has been studied histochemically.

2. Our results confirm that glycogen occurs in the decidua, the junctional zone trophoblast and the giant cells, and later in the visceral layer of the yolk sac and the labyrinthine syncytium.

3. PAS-positive diastase-fast materials, apparently of a glycoprotein nature, are widely distributed. Among those which have not been reported previously are large numbers of granules in the cytoplasm of the giant cells and, at 17 days, sparsely distributed fine granules in the labyrinthine syncytium.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A 12-day placenta showing the 'capsule' (1), the middle and inner zones of the decidua (2), the giant cell layer (3), the junctional zone (4), the labyrinth (5), the yolk sac (6) and the allantoic mesoderm (7). Trichrome. × 25.

Fig. 2. A decidual glycogen area in a 12-day placenta. PAS. × 650.

Fig. 3. The same area as shown in fig. 2 in an adjacent section. Diastase-PAS. × 650.

Fig. 4. A decidual glycogen area in a 17-day placenta. PAS. × 650.

Fig. 5. Glycogen in trophoblastic giant cells of a 12-day placenta. Dimedone-PAS. × 650.

Fig. 6. Cytoplasmic granules in trophoblastic giant cells. Diastase-PAS. × 650.

PLATE 2

Fig. 7. Glycogen in the junctional zone trophoblast of a 12-day placenta. Dimedone-PAS. × 650.

Fig. 8. A junctional zone glycogen area at 17 days. PAS. × 650.

Fig. 9. Glycogen in the labyrinth of a 14-day placenta. Dimedone-PAS. × 1200.

Fig. 10. The labyrinth of a 14-day placenta. Diastase-PAS. × 650.

Fig. 11. Glycogen in yolk sac villi at 17 days. Dimedone-PAS. × 650.

Fig. 12. The visceral yolk sac epithelium at 12 days. PAS. × 650.

PLATE 3

Fig. 13. The endovascular plasmodium in a 12-day placenta. Several granulated metrial gland cells can also be seen. Diastase-PAS. × 650.

Fig. 14. Cytoplasmic granules in trophoblastic giant cells. Coupled tetrazonium. × 850.

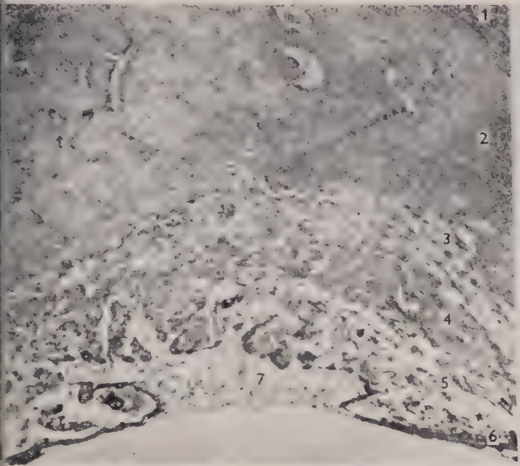
Fig. 15. Perinuclear granules of a 12-day trophoblastic giant cell. Sulphation-azur A. × 1500.

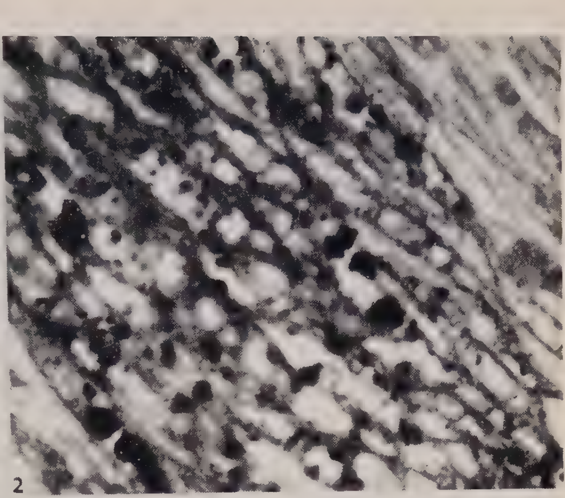
Fig. 16. The labyrinth of a 17-day placenta. Sulphation-azur A. × 650.

Fig. 17. The labyrinth of a 14-day placenta. Azo-dye method for alkaline phosphatase. × 750.

Fig. 18. The capsule situated between middle and outer zones of mesometrial decidua. Methylene blue at pH 3·75. × 650.

All the PAS preparations which are illustrated are lightly counter-stained with celestin blue.

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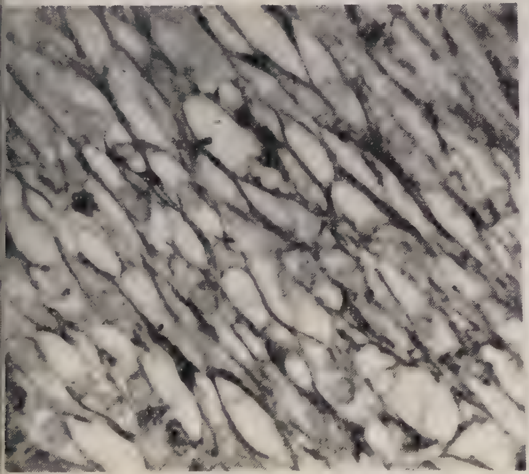

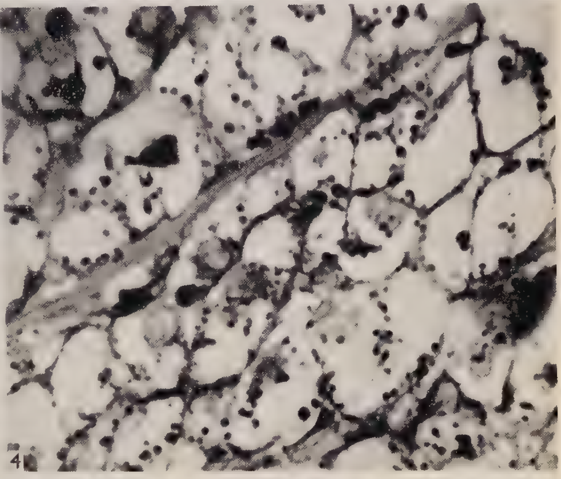
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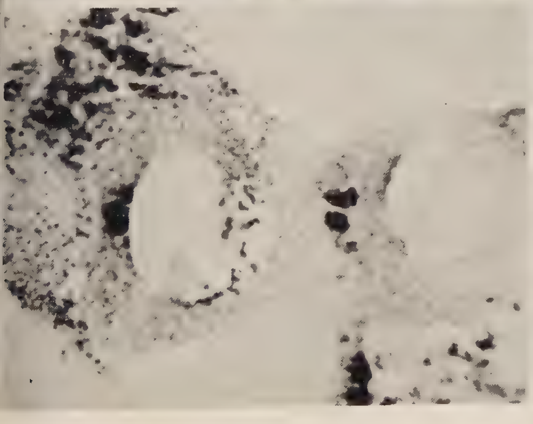

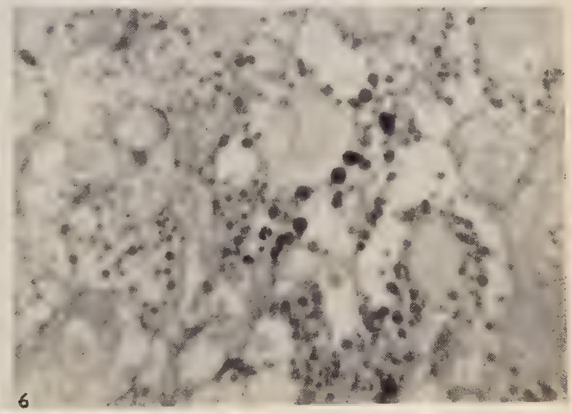
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BULMER AND DICKSON—OBSERVATIONS ON CARBOHYDRATE MATERIALS IN THE RAT PLACENTA

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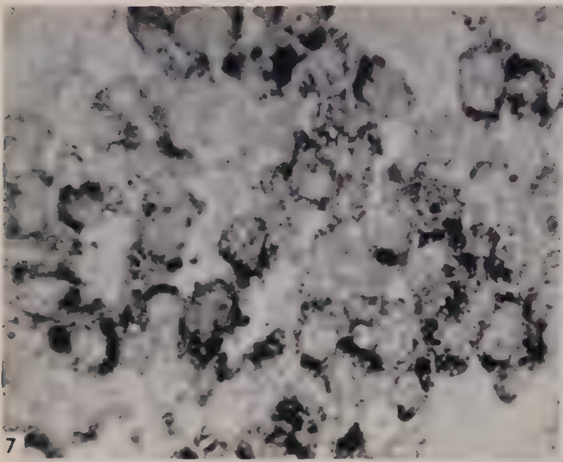
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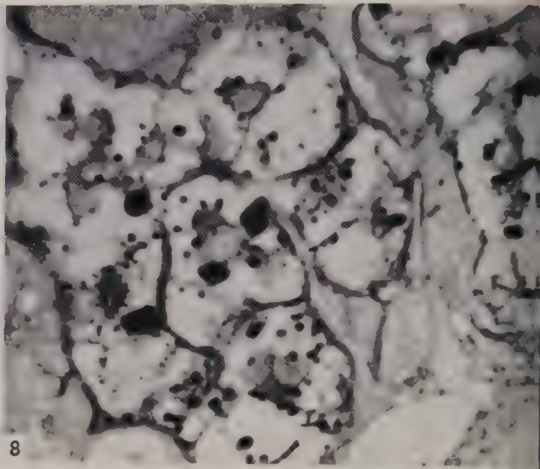
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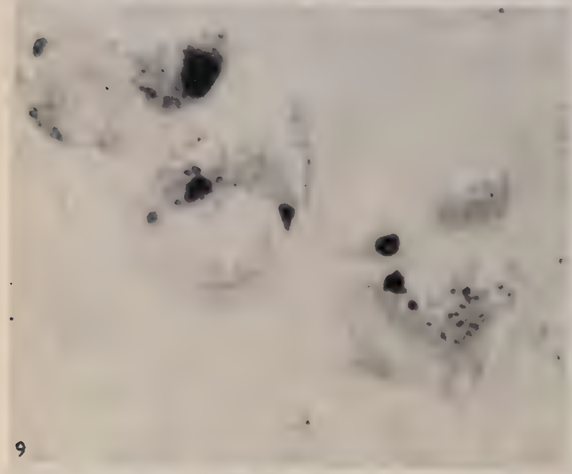
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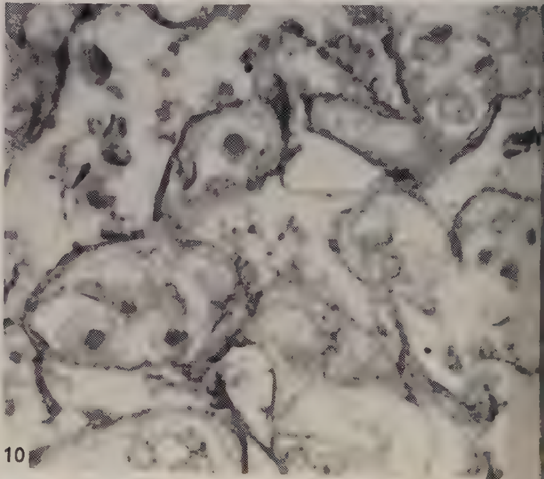
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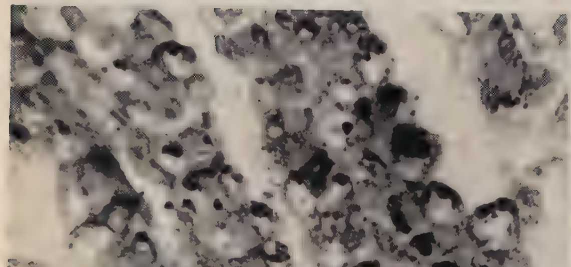
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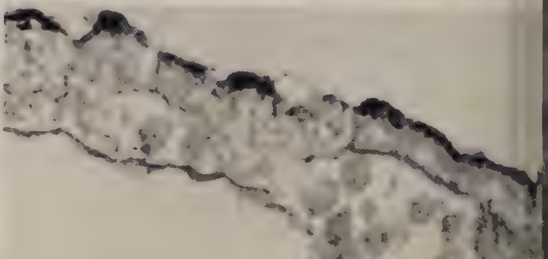
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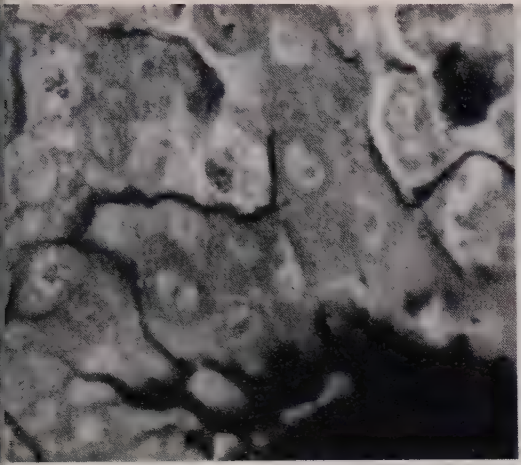
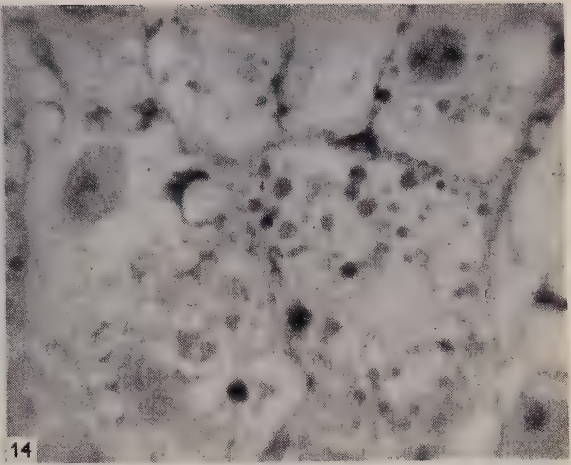
BULMER AND DICKSON—OBSERVATIONS ON CARBOHYDRATE MATERIALS IN THE RAT PLACENTA





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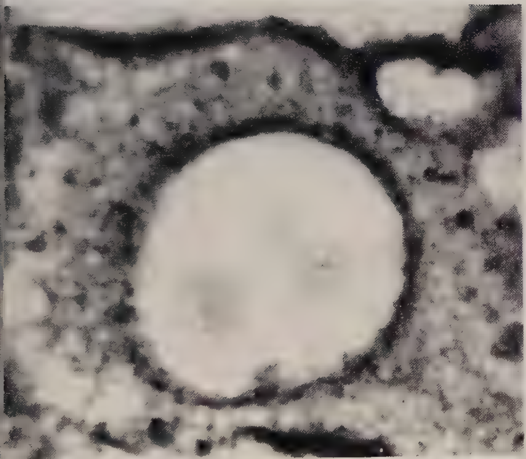

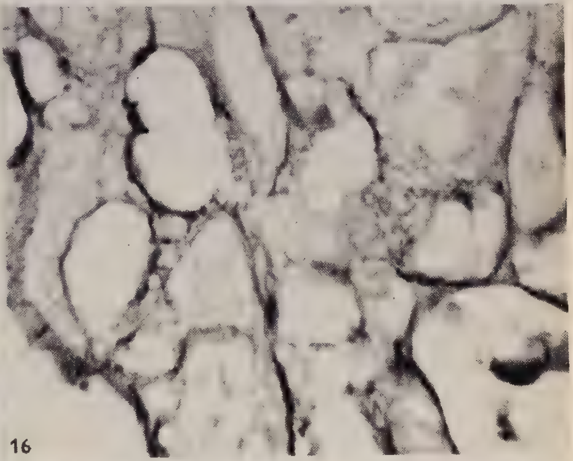

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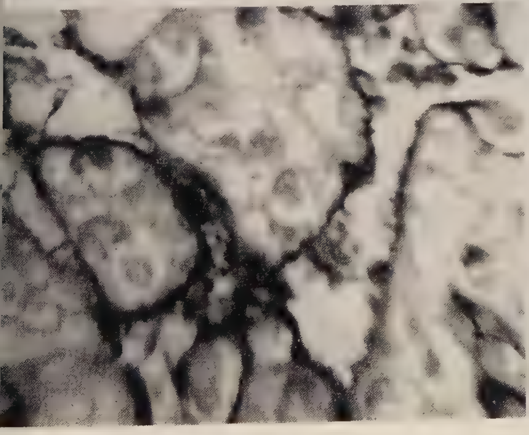

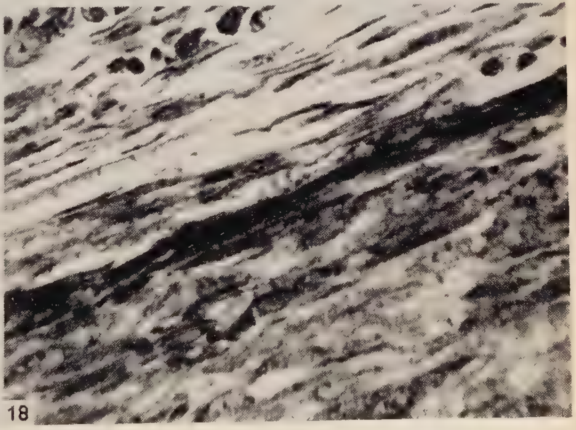

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BULMER AND DICKSON—OBSERVATIONS ON CARBOHYDRATE MATERIALS IN THE RAT PLACENTA

THE SALIVATORY CENTRE IN THE RAT

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INTRODUCTION

A centre for salivary secretion is situated, according to most accounts, in the lateral reticular formation of the hindbrain, in the junctional region between pons and medulla oblongata, at the level of, or just caudal to, the facial nerve nucleus. The nerve cells concerned are considered to fall into two groups: a superior salivatory nucleus, giving rise to preganglionic fibres which leave the brain stem in the nervus intermedius and supply the lacrimal, submandibular and sublingual glands; and an inferior salivatory nucleus, placed more caudally, which contributes a visceral efferent component to the glossopharyngeal nerve and furnishes the secretomotor supply to the parotid gland. This description is based mainly on reports of retrograde cellular degeneration after lesions involving the chorda tympani and the tympanic branch of the glossopharyngeal nerve. There is disagreement, however, as to the exact site of the nuclei and whether they project to the same or to the opposite side of the head. Bischoff (1899) originally noted in Marchi preparations of the cat's brain a crossed bundle which left the central nervous system with the vestibular root. Kohnstamm (1902*a*, *b*) concluded that this bundle formed part of the salivatory pathway, and that its fibres arose from a superior salivatory nucleus situated in the lateral reticular formation of the opposite side. Kohnstamm (1903) further described an inferior salivatory nucleus, concerned with the innervation of the parotid, situated in the medulla between the inferior olive and the nucleus ambiguus. Yagita & Hayama (1909) and Yagita (1910), using the method of retrograde degeneration in the dog, located the salivatory centre ipsilaterally in the lateral reticular formation. Kaida (1929) divided the chorda tympani in the rabbit and obtained a retrograde degeneration mainly on the operated side, but the Marchi method following a dorsal hindbrain lesion lateral to the facial genu revealed degenerating fibres in each chorda tympani. Kaida concluded that Bischoff's bundle contained a crossed and uncrossed salivatory outflow, and that twice as many fibres took the ipsilateral route. Rasmussen (1946) failed to obtain degeneration of the chorda tympani in cats after lesions of the reticular formation dorsal to the superior olive. His results confirmed some previous work by Papez (1930) indicating that Bischoff's bundle was a continuation of the peduncle of the superior olive. Rasmussen believed that the crossed fibres arose not from the superior olive itself, but from retro-olivary cells of visceral efferent type, and later (1953) he traced their course out of the brain stem with the eighth nerve to the spiral ganglion of the cochlea and beyond. Szentágothai (1952) produced Marchi degeneration of the chorda tympani in the cat with ipsilateral brain stem lesions which seemed to locate the superior salivatory nucleus between the motor and main sensory trigeminal nuclei—that is, more rostrally in the lateral reticular formation than other workers had supposed.

Finally, Torvik (1957) investigated the site of the salivatory centre by avulsing the facial nerve—nervus intermedius complex in kittens. Retrograde degeneration occurred ipsilaterally in the lateral reticular formation dorsal to the caudal part of the superior olive and the rostral pole of the facial nucleus, and extended caudally medial to the solitary tract.

Individual salivatory neurons are not readily recognizable in normal adult brain stained by the usual methods, and for this reason it has been suggested by some authors that they may not form compact nuclei, but instead are diffusely scattered amongst other cells of the reticular formation. Nevertheless, Lorente de Nó (1922) and Aström (1953) have both identified an area of the lateral reticular formation as the superior salivatory nucleus in silver preparations of the mouse, and have illustrated what they took to be salivatory cells. Furthermore, Kimmel (1940) has described the development of both the superior and inferior salivatory nuclei in the rabbit from a dorsal visceral efferent cell column. His reconstruction of the adult brain places the salivatory nuclei well caudal to the abducens nucleus, at the rostral end of the dorsal motor nucleus of the vagus.

Like the more recent anatomical studies, the electrophysiological evidence tends to favour the notion of an uncrossed salivatory outflow. Miller (1913) obtained ipsilateral salivary secretion on stimulating the floor of the fourth ventricle in cats, and described submandibular and parotid 'points' where secretion from these glands was maximal. He also induced reflex secretion by stimulating the chorda-lingual and the glossopharyngeal nerve on the same side. Chatfield (1941) recorded one instance of bilateral submandibular secretion on stimulating the brain substance lateral to the facial genu, and a number of instances of bilateral parotid secretion with more caudal stimulation. Magoun & Beaton (1942) and Wang (1943), working on monkeys and cats respectively, each obtained reactions which were almost entirely ipsilateral. Rostral stimulation yielded mainly submandibular responses and caudal stimulation parotid responses, with an area of overlap. The authors considered their results to be consistent with a salivatory centre sited either dorsomedially just caudal to the facial genu, or more ventrally in the lateral reticular formation, in which case the efferent fibres must first run dorsomedially to form a genu similar to that of the facial nerve. Wang favoured the second alternative—influenced by the work of Yagita and apparently by a misunderstanding of the views of Kimmel, who did not, as Wang claimed, locate the salivatory nuclei laterally in the rabbit. Wang also reported salivary secretion, which he intepreted as reflex, after stimulating the region of the solitary tract and its nucleus and the rostral end of the spinal trigeminal tract and its nucleus.

It is evident from this account that the literature contains sufficient discrepancies to justify Ranson & Clark's statement (1959) that the exact location of the salivatory nucleus is unknown. The same authors are sceptical of Kohnstamm's findings, on the grounds that his alleged salivatory nuclei are composed of large cells unlikely to be derived from the visceral efferent column, rather than of medium-sized cells such as are found in the dorsal motor nucleus of the vagus. This objection applies equally to the descriptions of salivatory cells given by Lorente de Nó and Aström. It is also fair to say that the course within the central nervous system followed by the preganglionic fibres subserving salivation and lacrimation is no less obscure than the site of their parent cells. This is indicated by the confusion which has existed between salivatory and cochlear efferent fibres and by the uncertainty as to whether a significant number of salivatory fibres cross the midline. We determined, therefore, to try to demonstrate salivatory nuclei and pathways in the rat's brain by using a histochemical technique for cholinesterases similar to that devised by Koelle & Friedenwald (1949). Koelle (1954) listed nuclei and fibre tracts in the rat exhibiting cholinesterase activity without mentioning salivatory centres or paths, but the work of Snell (1958) on the peripheral innervation of the salivary glands of the rat suggested that the Koelle method might be adapted successfully to show the origin within the central nervous system of the secretomotor fibres to these glands. The difficulties of tracing nerve fibres to their cells of origin in normal material are well known, and a histochemical method could only prove superior if it could be shown to possess some degree of specificity for the type of neuron under study. For this reason we thought it undesirable to explore possible sites for the salivatory centres in the hindbrain without first observing the staining reactions of the visceral efferent column as a whole, and comparing their appearance with that of other cell groups in the hindbrain and midbrain.

MATERIAL AND METHODS

Five male albino rats aged 6 months were used for the histochemical part of this study. The brains were removed after carbon monoxide asphyxia, the cerebral and cerebellar hemispheres cut away, and lengths of brain stem taken which in one instance comprised the midbrain and in four instances extended from the trigeminal outflow to the more rostral rootlets of the glossopharyngeal nerve in the hindbrain. The specimens were fixed in 10 % formol saline for 1 hr. at room temperature and then for a further 6–18 hr. at 4° C. They were then well washed in distilled water, after which serial transverse sections were cut on the freezing microtome. Alternate sections of the midbrain series were cut at 50μ and 100μ, one of the hindbrain series was cut at 40μ and the remainder at 50μ. Sections were transferred individually from the microtome knife to a plastic haemagglutination tray containing the incubation medium; in this way the serial order of the sections was preserved. Sections were incubated at 37° C.: one hindbrain series was incubated for $4\frac{1}{2}$–$5\frac{1}{2}$ hr. and the other four series for approximately 3 hr. Following incubation the sections were well washed in several changes of distilled water, treated with yellow ammonium sulphide solution for 2 min. and re-washed in water; after which they were mounted serially on glass slides, allowed to dry at room temperature and placed in a drying oven at 37° C. overnight. The slides were then placed in absolute alcohol for a few minutes and mounted from xylene in neutral balsam.

For the midbrain series and for three of the hindbrain series, the incubation medium contained: 4 mM. acetylthiocholine sulphate, 5 mM. copper sulphate, 25 mM. glycine, 25 mM. acetic acid, 50 mM. sodium acetate. The final pH of the solution was 4·9.

For one of the hindbrain series, cut at 50μ, five different incubation media were used (Table 1) containing inhibitors chosen because they have been found to give the greatest differential inhibition of the true and pseudo-cholinesterases in the rat's brain (Bayliss & Todrick, 1956). Apart from the change of substrate and the addition of inhibitors the solutions were identical with the standard medium.

The serial order was preserved, every fifth section being incubated in a particular medium.

One of the rats was used to obtain whole mounts of the seventh nerve—nervus intermedius complex. The nerve trunks, together with the geniculate ganglion, were detached from the brain, incubated in the standard substrate medium for 4–8 hr., very well washed in water, treated with ammonium sulphide and then mounted in glycerol for examination.

Table 1

Medium number	Substrate	Inhibitor
1	Acetylthiocholine	None
2	Acetylthiocholine	5×10^{-5} M 62C47
3	Acetylthiocholine	10^{-4} ethopropazine
4	Butyrylthiocholine	None
5	Butyrylthiocholine	10^{-4} ethopropazine

No counterstain was found necessary for any of the material stained in this way for cholinesterases. Nuclei and fibre tracts in the various transverse series were identified by comparison with serial transverse sections of rat's brains stained by Bodian's silver proteinate method, by a modification of Weigert's method for myelin, and with cresyl violet. In addition, use was made of two transverse neonatal series through the head region stained by Bodian's method after chelation with 'Versene'.

OBSERVATIONS

The course of the nervus intermedius outside the central nervous system was followed in both neonatal silver series. In each animal and on each side the single trunk of the nerve could be seen to divide proximal to the geniculate ganglion into a dorsal and a ventral root. Both roots could be traced to the point where they reached the surface of the brain stem, but no further: the dorsal root apparently followed the ventral border of the root of the vestibular nerve. The two roots of the nervus intermedius were also identified, with fibres deeply stained, in a whole mount of the nerve prepared by the Koelle method.

A teased whole mount of the nervus intermedius and facial nerve showed the fibres of the former to be deeply impregnated with copper sulphide, whereas the latter by comparison was very lightly stained. Similarly, in a transverse hindbrain series in which the seventh and eighth nerve roots had not become detached during the process of sectioning, the same differential staining could be seen, while the root fibres of the vestibular nerve were not picked out at all beyond the general background staining of the central nervous system (Pl. 1, fig. 6). The remarkably heavy staining of the roots of the nervus intermedius was found to continue in this and in all the other hindbrain series into the central nervous system, causing the nerve bundles to stand out clearly against the background. At the same time, it was evident that certain specific nuclei and other more diffuse groups of cells in the brain stem were also selectively stained. The relative affinity of different cell groups for the Koelle stain was approximately the same in all the series, but was much easier to assess when incubation had not been too prolonged. Moreover, in the sections which had been incubated for $4\frac{1}{2}$–$5\frac{1}{2}$hr., certain nerve cells were picked out—particularly the large cells of the lateral and superior vestibular nuclei of Deiters and Bechterew and large cells of the ventral reticular nucleus in the medial reticular formation—which in the other series were unstained or only slightly stained. For this reason, in searching for the salivatory nuclei, attention was directed especially towards the more critically stained specimens in which incubation was cut short after 3 hr. The thinnest series, sectioned at 40 μ, proved to be the most useful.

The following named nuclei of the hindbrain were found to stain after incubation with the standard substrate medium for 3 hr.: in the medulla, the dorsal motor nucleus of the vagus and the nucleus ambiguus; in the pons and ponto-medullary junctional region, the facial nucleus, the accessory facial nucleus (i.e. the small group of cells interposed between the main facial nucleus and the motor trigeminal nucleus, and alternatively known as the accessory abducens nucleus), the motor trigeminal nucleus, the nucleus of the locus coeruleus, the preolivary nucleus, and (rather faintly) the pontine nuclei. In the midbrain the following nuclei were found to stain, the nomenclature being that of Gillilan (1943): the Edinger-Westphal nucleus pars rostralis (nucleus medianus anterior) and pars caudalis; the red nucleus pars magnocellularis; the interpeduncular nucleus, median nucleus of the raphe, nucleus medialis profundus (ventral tegmental nucleus), dorsal tegmental nucleus and latero-dorsal tegmental nucleus; the rostral ventral nucleus of the lateral lemniscus, the nucleus of the basal optic root, and the stratum griseum intermediale of the superior colliculus. In some regions, such as the pars compacta of the substantia nigra and the stratum griseum superficiale of the superior colliculus, the staining was diffuse and appeared to be located outside the cells, possibly in nerve terminals. The somatic motor nuclei—hypoglossal, abducens, oculomotor and trochlear (the last two are continuous in the rat)—were readily recognizable, although their cells were only lightly stained. By comparison, the branchiomotor nuclei of the fifth and seventh nerves were moderately stained, while the nucleus ambiguus was made very conspicuous by a considerable extracellular deposit superimposed on the intracellular staining. Staining was heavy in the visceromotor column (Edinger-Westphal nucleus and dorsal motor nucleus of the vagus) and in the nucleus of the locus coeruleus, and appeared to be almost entirely intracellular, so that the individual cells were clearly delineated.

In the hindbrain series for which different incubation media were used, the nuclei enumerated above were stained in all sections which had been incubated in media containing the acetyl substrate (media 1, 2 and 3). The intensity of staining was approximately equal in sections which had been incubated in medium 1 (without inhibitor) and in medium 3 (containing inhibitor for pseudo-cholinesterase) and was reduced in sections incubated in medium 2 (containing inhibitor for true cholinesterase). In sections incubated in medium 3, the cells constituting the various nuclei were more clearly differentiated from their background, and the staining of blood vessels was eliminated. The equivalent nuclei were unstained in those sections which had been incubated in media containing the butyryl substrate (media 4 and 5) which is not hydrolysed by true cholinesterase. Sections incubated in medium 5 were entirely blank: those incubated in medium 4 were blank except for blood vessels and a scattering of small deeply stained cells in the caudoventral pole of the main sensory trigeminal nucleus, where it is pierced by the ventral root of the nervus intermedius.

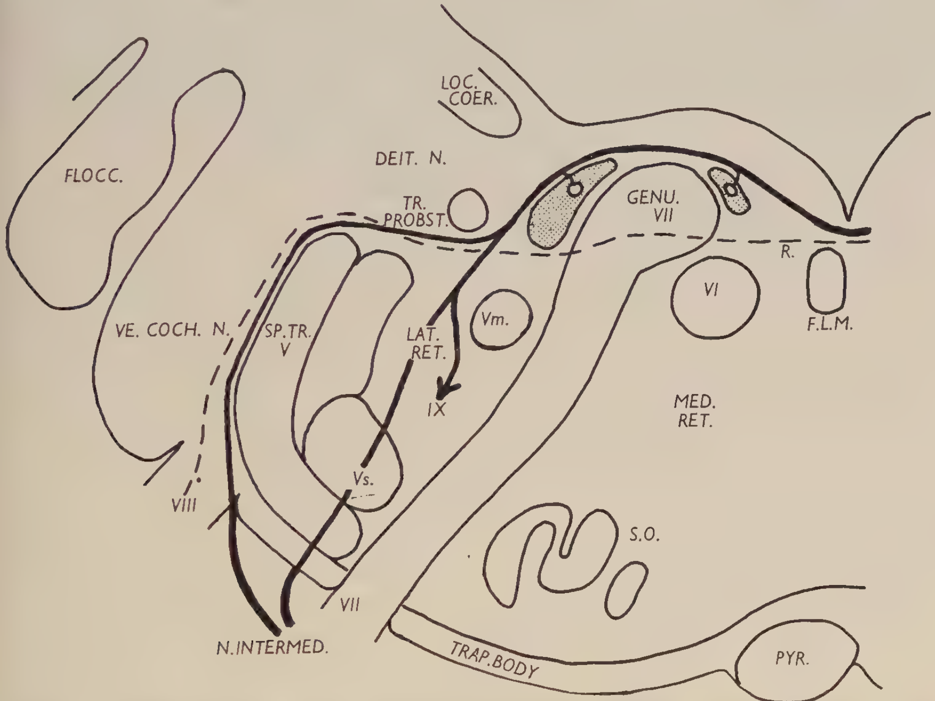
Cells in the same position were a conspicuous feature of sections incubated in media 1 and 2, but were absent in sections incubated in medium 3. In other words, the enzyme responsible for the staining properties of these cells, which have the appearance of being neurons, was pseudo-cholinesterase and not true cholinesterase.

In all sections stained for true cholinesterase, the two roots of the nervus intermedius were clearly distinguishable. They were followed throughout their extent in the three hindbrain series which had been incubated in the standard substrate medium. The ventral root could be seen to take a direct course from the region of the genu of the facial nerve ventrally, laterally and caudally through the lateral reticular formation, and to emerge from the brain stem by piercing successively the caudo-ventral pole of the main sensory trigeminal nucleus, the ventral part of the spinal trigeminal tract, and the trapezoid body (Pl. 1, fig. 5). The dorsal root arched laterally from the same region, below the lateral vestibular (Deiter's) nucleus, over the lateral reticular formation, the main sensory trigeminal nucleus and the spinal trigeminal tract, then turned ventrally in company with the root fibres of the vestibular nerve (Pl. 1, fig. 4), to emerge lateral and rostral to the ventral root. Parts of the ventral root and the horizontal portion of the dorsal root were recognizable in the silver and Weigert material. The horizontal fibres of the dorsal root could be seen to pass mainly below, although a few intersected, the bundle constituting the tract of Probst which descends from the mesencephalic trigeminal nucleus.

Proximally, the two roots of the nervus intermedius were found in the Koelle sections to split from a common trunk. This trunk emerged from a collection of nerve cells flanking the lateral aspect of the facial genu at its rostral end, where the facial nerve fibres turn down to form the descending limb of the facial root (Pl. 1, fig. 2). A number of fibres continued over the dorsum of the facial genu to a smaller collection of cells on its medial aspect, and a few fibres crossed the midline immediately below the floor of the IVth ventricle to join the equivalent cells of the opposite side (Pl. 1, fig. 1). The cells of both groups were heavily stained, and in addition there was a considerable extracellular deposit, which caused them to form conspicuous nuclei in transverse sections, although the larger was only 300μ long. The clarity of the picture at this level, due to the surrounding nuclei and fibre tracts failing to stain, was of assistance in deciding whether the association between the nervus intermedius and the hitherto undescribed nuclei related to the facial genu was a fortuitous one. No individual fibres of the nervus intermedius could be traced to cells in any other situation, and no other stained cell group lay on the main course of the nerve. On the assumption, therefore, that the nuclei on either side of the facial genu were in fact the source of the stained fibres in the nervus intermedius, they will hereafter be referred to in this paper as the *lateral* and *medial salivatory nuclei.* They were unstained in sections incubated in media 4 and 5 containing the butyryl substrate, and so presumably contain only true cholinesterase.

The Koelle method failed to stain any cells of the lateral reticular formation in the traditional site for the salivatory nuclei and no distinct 'inferior' salivatory nucleus was found. There was, however, a possible route by which secretomotor fibres might reach the parotid from the lateral and medial salivatory nuclei, since some fibres from the ventral root of the nervus intermedius emerged from the brain stem at a more caudal level with the ventral rootlets of the glossopharyngeal nerve.

The ventral glossopharyngeal and vagal rootlets were themselves stained, and many of the stained fibres could be traced from the region of the dorsal motor nucleus of the vagus. The more dorsal glossopharyngeal rootlets, which could be seen in silver and Weigert material to form the bulk of the glossopharyngeal nerve and to contribute to sensory structures (tractus solitarius and spinal trigeminal tract), were not stained in the Koelle sections. The location of the salivatory nuclei and the course taken by the two roots of the nervus intermedius are illustrated in relation to other hindbrain structures in Text-fig. 1.



Text-fig. 1. Cross-sectional diagram, semischematic, of the junctional region between pons and medulla, showing the site of the lateral and medial salivatory nuclei (stippled), and the course of the dorsal and ventral roots of the nervus intermedius (heavy black). Rasmussen's crossed olivocochlear bundle is indicated by a broken line.

The cells of the lateral and medial salivatory nuclei could scarcely be distinguished in adult material stained by Bodian's method or cresyl violet from those of the adjacent grey matter, even though their site was known. No difficulty was found, however, in the neonatal specimens, and both nuclei were identified in their characteristic position relative to the facial genu, ventral to the caudal pole of the nucleus of the locus coeruleus. As in the adult, the lateral and medial nuclei were formed by distinct groups of cells, of which the lateral was the larger. The cells were multipolar, small or medium sized, and often contained two nucleoli within the nucleus (Pl. 1, fig. 3).

In addition to the few salivatory fibres which cross between the facial colliculi, the Koelle method made evident a more massive decussation in this region (Pl. 1, fig. 9), which could be seen in silver and Weigert sections to consist of fine, closely packed, lightly myelinated fibres. In the Koelle sections which received $5\frac{1}{2}$ hr. incubation, some fibres could be followed from the facial genu to the dorsal root of the nervus intermedius (Pl. 1, fig. 8). These fibres accompanied those of the dorsal root of the nervus intermedius throughout its course within the central nervous system, but emerged in the substance of the vestibular root of the auditory nerve (Pl. 1, fig. 7). They were less heavily stained than the fibres of either root of the nervus intermedius, but could be clearly seen within the vestibular root, since the latter was unstained. In the material available to us, these fibres could not be traced further than the vestibular ganglion. The decussating fibres, and the fibres accompanying the dorsal root of the nervus intermedius and leaving the brain stem with the vestibular root, occupied positions which corresponded exactly to the course attributed by Rasmussen (1946) to his olivocochlear bundle.

DISCUSSION

The present work represents an attempt to solve a neurological problem by histochemical means. In assessing the validity of this approach, it is necessary to give some consideration to the rationale of the method as used by us and by other workers. In essence, the Koelle technique aims to show the presence of true cholinesterase in the cell bodies and fibres of neurons which can be regarded as cholinergic (Koelle, 1951, 1955). When our findings are compared with the total list of midbrain and hindbrain structures stated by Koelle (1954) to show specific (true) cholinesterease activity, it becomes evident that using a more acid pH without prolonging excessively the incubation time has the effect of increasing the selectivity of the substrate solution. This, for our purposes, had the desired result of causing certain fibre bundles and cell groups to stand out with exceptional clarity, so that they could be easily followed in serial sections. In so doing, we may have failed to stain some neurons, particularly their fibre processes, which nevertheless contain small amounts of enzyme. In point of fact, most of the structures found by Koelle to stain moderately or lightly with his more alkaline pH remained unstained in our sections. Apart from a few exceptions (such as the nucleus of the locus coeruleus and the red nucleus which, according to Koelle, were respectively moderately and lightly stained) the nuclei picked out by our technique were those classified by Koelle as exhibiting intense cholinesterase activity. Since the staining of all these nuclei, other than the scattered cells in the caudoventral pole of the main sensory trigeminal nucleus, is markedly reduced by a true cholinesterase inhibitor and is absent under conditions designed to show only pseudo-cholinesterase, it may be presumed that they at least are rich in true cholinesterase and consist of cholinergic neurons.

It may be asked why, in what was intended to be a complete survey of the cholinesterase activity of the rat's brain, Koelle did not report staining of the cell groups which we observed on either side of the facial genu. Since these nuclei stained heavily with us, it does not seem likely that they would fail to stain with what is in general a less selective method. Possibly Koelle overlooked them, as relatively minor collections of cells not mentioned by the authorities which he cited as having formed the source of his identifications. Alternatively, the fact that the much larger abducens nucleus is also missing from his list may be an indication that Koelle's material was defective in the crucial region. The reasons for regarding these nuclei as salivatory may be summarized as follows: they occur on the central course of what may be taken to be the secretomotor component of the nervus intermedius; no other nerve fibres could be detected in direct relation to fibres of the nervus intermedius; a location for the salivatory centre here is in accordance with physiological (though not with previous anatomical) findings; no cells stained in the lateral reticular formation where most other authors have placed the salivatory nuclei. In an attempt to establish our contention, these four points will be taken in order and discussed further.

(1) It was fortunate for the method by which we chose to approach our problem that the fibres of the nervus intermedius stained more heavily throughout their course than any other fibre tract encountered. This may have been partly due to their fine calibre, causing a given quantity of enzyme per neuron to be more concentrated within the fibre, or to the compactness of the bundles into which they are aggregated, or perhaps to some intrinsic property of salivatory fibres. Our material offers some evidence that different types of cholinergic neuron may have different staining affinities, since visceromotor neurons stained on the whole more heavily than branchiomotor neurons, and the latter more heavily than somatic motor ones. It seems fairly certain that the fibres of the nervus intermedius which took the stain were in fact its preganglionic secretomotor component. Snell (1958) detected a compact group of fibres rich in cholinesterase in the chorda tympani of the rat, but since he also found collections of ganglion cells along their course, it is not clear whether the fibres at this level were preganglionic or postganglionic. It is unlikely that any of the stained fibres of the nervus intermedius would be sensory, since the solitary tract was never stained in our sections. This is in agreement with the general rule that fibres of first sensory neurons do not contain a high concentration of cholinesterase (Koelle, 1951). The main contribution to the solitary tract in the rat can be seen in silver and Weigert material to come from dorsal rootlets of the glossopharyngeal and vagal nerves. These show no cholinesterase activity, and are presumably purely sensory. They appear to be in series with the dorsal root of the nervus intermedius, which possibly carries in addition to its motor component a few sensory fibres to the rostral end of the solitary tract. The ventral root of the nervus intermedius seems to be purely secretomotor, and in series with the ventral rootlets of the glossopharyngeal and vagal nerves, which were interpreted as motor by Tarlov (1940). The deeply stained fibres of the nervus intermedius can be traced as far as, but not beyond, the cell groups which we have described as lateral and medial salivatory nuclei. Since these nuclei are themselves rich in cholinesterase, the natural inference is that they are not fortuitously associated with the trunk of the nervus intermedius, but in fact form the source of its secretomotor fibres. Whether or not they comprise the only lower salivatory centre requires more consideration.

(2) If any stained fibres of the nervus intermedius arise from cell bodies other than those of the lateral and medial salivatory nuclei, their course before joining the main trunk of the nervus intermedius must be so dispersed as to cause the individual fibres to escape detection. Although in sections prepared by the Koelle technique it is much easier to spot aggregations of nerve fibres than fibres running in isolation, yet in favourable instances single fibres can be seen, especially with slides which have

been stored for several weeks, when the background staining begins to fade. We found no evidence of scattered fibres converging on the trunk of the nervus intermedius in our material. Another possibility is that two types of secretomotor fibre run in the nervus intermedius: one type, rich in cholinesterase, arising from the lateral and medial salivatory nuclei; and a second, low in enzyme, arising from some other source. We are reluctant to adopt a double hypothesis of this sort unless the evidence for it, physiological and anatomical, can be shown to be very strong.

(3) The salivatory secretion obtained by Wang (1943) in the cat, and by Magoun & Beaton (1942) in the monkey, would be fully explained by the presence of a dorsomedial salivatory centre related to the genu of the facial nerve, in a position similar to that of the lateral and medial salivatory nuclei of the rat, and does not demand—although it does not absolutely preclude—a ventrolaterally placed centre in the lateral reticular formation. Our observation that only a few fibres belonging to the trunk of the nervus intermedius cross between the two medial salivatory nuclei supports the conclusion of these authors that the salivatory outflow is predominantly homolateral. The existence of a partial decussation of preganglionic neurons would account for the scanty secretion from contralateral glands which they obtained after stimulation close to the midline dorsally. The physiological evidence also supports our contention that there is no separate 'inferior' salivatory centre for parotid secretion. The distribution maps of points in the hindbrain yielding salivary flow show a concentration of points anteriorly around the facial genu; the spread of points in the lateral reticular formation is a linear one, such as might be expected to result from stimulation of the roots of the nervus intermedius and the fibres which join the motor root of the glossopharyngeal nerve, rather than from the activation of separate centres. Indeed, the general accord with the physiological results of Wang and of Magoun & Beaton shown by the present anatomical findings suggests that they may not be limited in their application merely to rats, but may prove to have a more general validity for mammals as a whole. The implication, following upon the apparent absence of a genu on the course of the nervus intermedius, would be that the salivatory nucleus of mammals retains its primitive dorsomedial position on the visceral efferent column, and that its cells do not undergo a ventrolateral migration like those of the facial nucleus during ontogeny. The division into lateral and medial nuclei may be secondary to the development of the genu of the facial nerve.

(4) Amongst the possible sites for salivatory cells in the rat must be included the supratrigeminal nucleus, which Lorente de Nó (1922) regarded as forming the rostral end of the salivatory column. Since this nucleus did not appear stained in the Koelle sections, it may, as Torvik (1956) suggested, consist rather of internuncial neurons on a trigeminal reflex pathway, receiving collaterals from the mesencephalic trigeminal tract. A more serious problem is set by the various areas of the lateral reticular formation which have been said to suffer a retrograde cellular degeneration after injury to the salivatory outflow, and the question arises whether our failure to demonstrate staining anywhere in the lateral reticular formation is sufficiently conclusive to rule this region out as a site for salivatory nuclei. In attempting to decide this point, it is relevant to consider the likely source of the cholinesterase present in the axons of cholinergic nerves. Sawyer (1946) sectioned the sciatic nerve in guinea-pigs and estimated the enzyme content of degenerating and re-

generating fibres, and found it to fall in the former by about 60 % and increase in the latter by 3 times within 10–14 days. He concluded from his figures that nearly two-thirds of the true cholinesterase contained in the nerve is secreted by the axis cylinder, and that more than one-third is produced elsewhere—possibly by the sheath cells. Hebb & Waites (1956) obtained a similar distribution of choline acetylase following section of the cervical sympathetic nerve in sheep, and pointed out that their results and Sawyer's would be most easily explained on the assumption that the enzymes secreted by the regenerating axis cylinders were originally derived from the cell body. There is an increasing body of evidence from other sources to support the hypothesis that the cholinesterase contained within an axon is synthesized in the body of its parent cell, and we therefore think it most unlikely that fibres which stain as heavily as do those of the nervus intermedius in the rat could arise from nerve cells which by the same technique do not stain. This view conforms with the generalization propounded by Koelle (1951), that true cholinesterase is present in high concentration throughout the cell bodies, dendrites and axons of cholinergic nerves, including preganglionic neurons of the autonomic nervous system.

If it be accepted that the present results point definitely to the lateral and medial salivatory nuclei as the sole source of preganglionic salivatory and lacrimatory fibres, there remains the problem of how to reconcile this conclusion with the discrepant findings of previous workers, and particularly with the reports of cellular degeneration in the lateral reticular formation. Chromatolysis following nerve section is not always a very clear-cut phenomenon, and it is generally recognized now that even in practised hands the method may be fallible. If degeneration truly followed lesions of the nervus intermedius, chorda tympani or superficial petrosal nerves, it may possibly have been trans-synaptic, involving neurons of the lateral reticular formation projecting on to the salivatory nuclei. In cases where the lesion included the facial nerve, some of the degenerating cells may have belonged to the accessory facial nucleus. In our Koelle sections, the staining of this nucleus was moderately heavy, approximating in intensity to that of the motor trigeminal and facial nuclei. This observation is in line with the claims made by Szentágothai (1948, 1949) that the accessory facial nucleus is branchiomotor and supplies the posterior belly of the digastric muscle, as against the view that is is related to the abducens nucleus, the cells of which were only lightly stained.

The heavy intracellular staining of the lateral and medial salivatory nuclei was comparable in intensity to that of the other elements of the visceral efferent column —namely the dorsal motor nucleus of the vagus and the nucleus of Edinger-Westphal—but differed from them and resembled the nucleus ambiguus in that a considerable amount of extracellular stain was also present. The significance of such dense deposits of true cholinesterase outside the cell is not clear. One possibility is that the enzyme has been extruded from inside the cell body; another is that it has been released from cholinergic terminals synapsing on the cell. The intense staining of the small cells constituting the nucleus of the locus coeruleus, contrasting with the large unstained unipolar cells of the adjacent mesencephalic trigeminal nucleus, and the dorsal position of the nucleus between the Edinger-Westphal and salivatory nuclei, suggests that the nucleus of the locus coeruleus may be yet another derivative of the visceral efferent column. An autonomic function for some cells of the mesencephalic trigeminal–locus coeruleus complex was postulated by Lewy, Groff & Grant (1938), although this was subsequently denied by Corbin & Harrison (1940).

The lateral and medial salivatory nuclei lie at the base of the area designated as the supragenual nucleus in the rabbit by Meessen & Olszewski (1949). Conspicuous though they are in Koelle sections, these groups of cells are extremely elusive in adult material prepared by conventional methods, so that it is not surprising that they should have escaped notice in the past. Cells of the lateral nucleus especially may on occasions have been ascribed to the abducens nucleus, and perhaps account for the small- and medium-celled ventricular portion of Fuse, discussed by Kappers, Huber & Crosby (1936). Kimmel (1940, 1941) described a collection of small reticular cells alongside the abducens nucleus in rabbits which, judging by its relations to the facial genu and the nervus intermedius, may have been the equivalent of one or other of our salivatory nuclei. Kimmel, however, did not regard these cells as visceromotor, but held rather that they formed the rostral end of a column of cells which included the nucleus praepositus and nucleus intercalatus, and received terminals of the afferent component of the nervus intermedius. The cell groups which he interpreted as superior and inferior salivatory nuclei appear to have lain a long way caudal to the salivatory nuclei described by us, and may have belonged to the rostral end of the dorsal motor nucleus of the vagus.

The confusion which seems to have existed between salivatory and cochlear efferent pathways is clarified on our interpretation of Bischoff's bundle, which is that it consists of the predominantly uncrossed dorsal root of the nervus intermedius accompanied by crossed fibres of Rasmussen's olivocochlear bundle. This bundle is evidently present in the rat, and its cholinesterase content, though apparently somewhat lower than that of the salivatory fibres, is such as to suggest that its fibres too may be cholinergic. The origin of the bundle could not be certainly determined, but no cells stained in the retro-olivary region which Rasmussen believed to be the main source of the olivocochlear fibres. A subsidiary source considered by Rasmussen (1946) was the medial preolivary nucleus. The cells of the (medial) preolivary nucleus—there is no distinct lateral preolivary nucleus in the rat—stain heavily in our material, in contradistinction to other parts of the superior olivary complex which are unstained. If, therefore, the crossed projection to the inner ear discovered by Rasmussen has a localized origin in the region of the superior olive, the preolivary nucleus would appear to be the most likely site.

SUMMARY

1. A modification of the Koelle technique for cholinesterases has been applied to the brain stem of the rat, and shown to be suitable for elucidating the salivatory pathways.

2. The nervus intermedius leaves the brain stem by two roots. The ventral root appears to be purely secretomotor, and makes a contribution to the glossopharyngeal nerve which is presumably preganglionic to the parotid. The dorsal root is accompanied by crossed fibres of Rasmussen's olivocochlear bundle and may carry a few sensory fibres to the tractus solitarius.

3. The roots of the nervus intermedius spring from two cell groups, the lateral

and medial salivatory nuclei, which flank the anterior end of the genu of the facial nerve root. The lateral nucleus is the larger. There is no 'inferior' salivatory nucleus associated exclusively with the glossopharyngeal nerve, and no evidence of salivatory cells in the lateral reticular formation.

4. The salivatory outflow is predominantly uncrossed. The decussation at the level of the facial colliculi is mainly formed by Rasmussen's bundle.

5. Other nuclei of the mid- and hindbrain which stain by the same method are mentioned. In most instances the enzyme present is true cholinesterase, but scattered cells in the caudoventral pole of the main sensory trigeminal nucleus contain pseudo-cholinesterase.

We wish to thank Prof. J. D. Boyd for the interest which he has taken in this work, also Mr H. Williams for technical assistance and Mr J. F. Crane for the photomicrography. We are further indebted to Messrs May and Baker Ltd. and to the Wellcome Research Laboratories for gifts of inhibitors.

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EXPLANATION OF PLATE

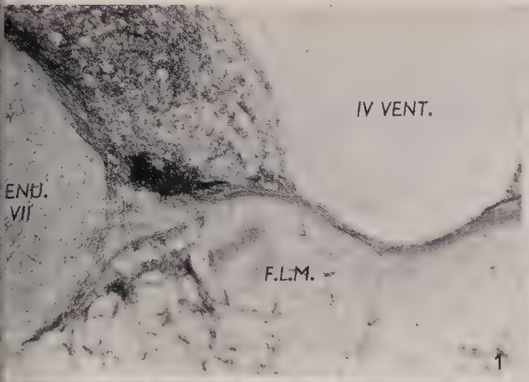
Key to abbreviations used in Text-fig. 1 *and Pl.* 1

DEIT.N.	Lateral vestibular nucleus of Deiters	*SP.TR.V.*	Spinal trigeminal tract
F.L.M.	Medial longitudinal bundle	*TR.PROBST.*	Descending mesencephalic tract of Probst
FLOCC.	Flocculus	*TRAP.BODY*	Trapezoid body
GENU VII.	Internal genu of facial nerve	*VE.COCH.N.*	Ventral cochlear nucleus
LAT.RET.	Lateral reticular formation	*IV VENT.*	Fourth ventricle
LOC.COER.	Nucleus of locus coeruleus	*Vm.*	Motor trigeminal nucleus
MED.RET.	Medial reticular formation	*Vs.*	Main sensory trigeminal nucleus
N.INTERMED.	Nervus intermedius of Wrisberg	*VI.*	Abducens nucleus
PYR.	Pyramid	*VII.*	Facial nerve
R.	Olivocochlear bundle of Rasmussen	*VIII.*	Vestibular division of auditory nerve
S.O.	Superior olive	*VIIIg.*	Vestibular ganglion

All sections photographed in Pl. 1 have been cut in the transverse plane.

Fig. 1. Medial salivatory nucleus, heavily stained. Koelle, 3 hr. incubation, 40μ, × 60.

Fig. 2. Lateral salivatory nucleus, heavily stained. Koelle, 3 hr. incubation, 40μ, × 60.



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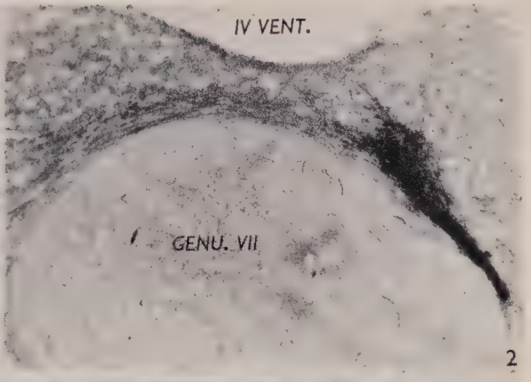

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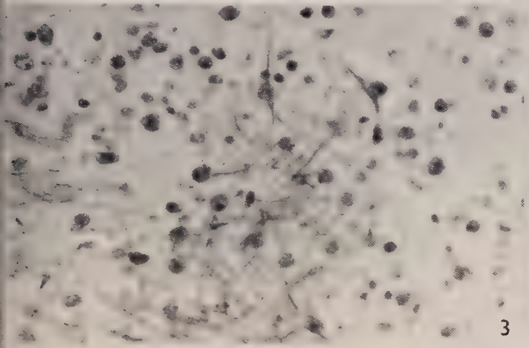

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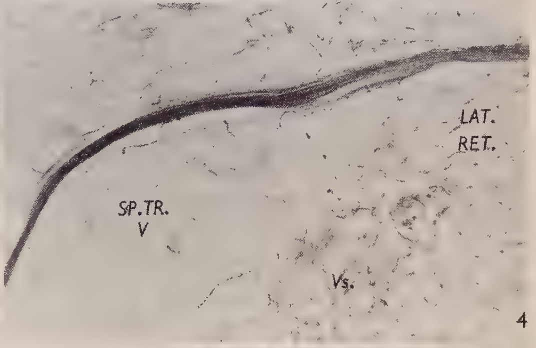

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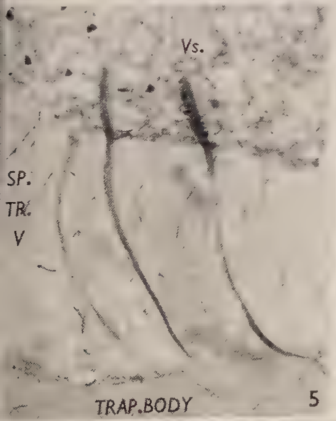

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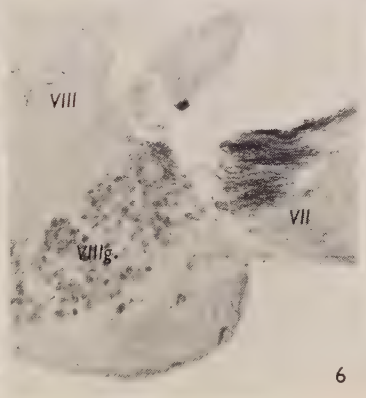

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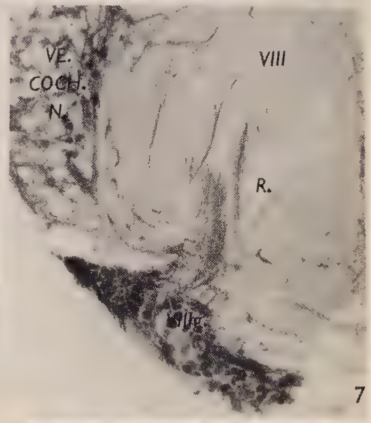

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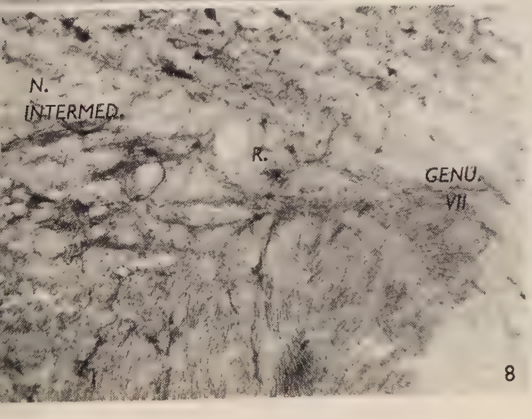

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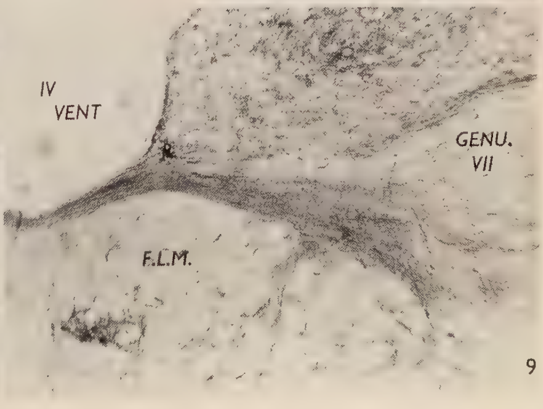

9

SHUTE AND LEWIS—THE SALIVATORY CENTRE IN THE RAT

Fig. 3. Lateral salivatory nucleus in neonatal rat. Bodian, 10 μ, × 270.

Fig. 4. Dorsal root of nervus intermedius, traversing lateral reticular formation and arching over spinal trigeminal tract. Koelle, 3 hr. incubation, 40 μ, × 60.

Fig. 5. Ventral root of nervus intermedius: rootlets traversing caudoventral pole of main sensory trigeminal nucleus and spinal trigeminal tract. Some pseudo-cholinesterase-containing cells visible in caudoventral pole of main sensory nucleus. Koelle, 3 hr. incubation, 40 μ, × 60.

Fig. 6. Nervus intermedius fibres heavily stained, facial nerve fibres lightly stained, vestibular nerve fibres unstained. Koelle, 4½ hr. incubation, 50 μ, × 60.

Fig. 7. Fibres of Rasmussen's bundle in vestibular root entering vestibular ganglion. Koelle, 5½ hr. incubation, 50 μ, × 60.

Fig. 8. Fibres of Rasmussen's bundle leaving facial genu to join dorsal root of nervus intermedius. Koelle, 5½ hr. incubation, 50 μ, × 60.

Fig. 9. Decussation at level of facial colliculi. Koelle, 3 hr. incubation, 40 μ, × 60.

HISTOCHEMICAL LOCALIZATION OF CHOLINESTERASE IN THE AMPHIBIAN SPINAL CORD AND ALTERATIONS FOLLOWING VENTRAL ROOT SECTION*

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The distribution of cholinesterases has been investigated in the spinal cord of various mammalian species, and the grey matter generally found to yield a positive histochemical reaction for the 'specific' enzyme (acetylcholinesterase, AChE), sometimes confined to motor nuclei in the ventral and lateral horns (Koelle & Friedenwald, 1949; Koelle, 1950, 1951, 1954, 1955; Hard & Peterson, 1950; Gerebtzoff, 1953; Ravin, Zacks & Seligman, 1953; Giacobini & Holmstedt, 1958). Sites of maximum AChE activity were detected in the cytoplasm or surface membrane of motor nerve cells and neurites, or in their immediate vicinities in the neuropil. In contrast to the 'neural' localization of AChE in the mammalian spinal cord, 'non-specific' cholinesterase (ChE) was found in non-neural structures, with the exception of some motor neurons exhibiting both enzyme activities (Koelle, 1954).

Besides the purpose of obtaining comparative histochemical data on the amphibian spinal cord, the present study appeared to be of interest in view of a feature described originally by Lavdowsky (1891), Sala (1892) and Cajal (1911), and recently reinvestigated with other techniques (Cerf & Chacko, 1957, 1958). In lower vertebrates, numerous branching expansions extend peripherally from the polar dendrites of the motoneurons (and from some interneurons), infiltrating the ventral and lateral columns and terminating in a dense marginal net under the pia. This 'perimedullary plexus', or 'lateral neuropil' receives axonal contributions constituted mainly by collaterals from the white matter and axons of some commissural cells, although the presence of motor axon and primary afferent collaterals has also been reported (Cajal, 1911; Sala, 1892; Kappers, Huber & Crosby, 1936; Silver, 1942*a*). Particular attention was given to this and other neuropil areas of the spinal cord, in view of the fact that a relatively high concentration of AChE has been noted histochemically in parts of the central nervous system where synaptic fields are well developed. In frog brain, for example, Shen, Greenfield & Boell (1955) reported the localization of

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the enzyme in centres rich in interneuronal connexions and poor in cellular elements, except for the motor nuclei of cranial nerves.

Further, alterations in the distribution of cholinesterase in the spinal cord after ventral root section were investigated. This procedure has been shown to initiate a retrograde reaction in motoneurons, which extends as far as the dendritic terminations in the perimedullary plexus (Cerf & Chacko, 1958).

MATERIAL AND METHODS

The bullfrog (*Rana catesbeiana*) and common toad (*Bufo boreas halophilus*) were used in this study. The spinal cord was removed from the decerebrated animal after perfusion of the vascular system with saline. Since the cholinesterase of amphibian tissues seems easily destroyed by ordinary fixation procedures (Couteaux & Taxi, 1952; Chessick, 1954; Shen *et al.* 1955), the lumbar enlargement was rapidly sliced into pieces 3–5 mm. thick and immediately lowered in liquid nitrogen. Fresh-frozen transverse sections (20 μ) were cut with the microtome placed in a cryostat kept at -10 to -15° C., and allowed to thaw on slides just before further processing. The histochemical method for detection of cholinesterases, using acetylthiocholine (AcThCh) or butyrylthiocholine (BuThCh) as substrates, was essentially similar to Koelle's improved technique (1951). Incubation in the presence of the following inhibitors was used to confirm the identity of the esterase at the site of enzymic reaction: (*a*) DFP (di-*iso*propyl-fluorophosphate), known as a potent inhibitor chiefly of cholinesterases other than AChE (Hawkins & Mendel, 1947; Koelle, 1950); (*b*) Burroughs and Wellcome B.W. 284 ^{51}C [(1-5-bis-4-allyl dimethylammonium phenyl) = pentan-3-one dibromide; Austin & Berry, 1953] found to be a highly selective inhibitor of AChE (Koelle, 1955). Appropriate concentrations were prepared from stock solutions of 10^{-1} M DFP in propylene glycol stored in the desiccator, and 10^{-2} M B.W. 284 kept in the refrigerator. The sections were divided in four groups, and incubated for 2 hr. at 37° C. in the following media, respectively: group I: AcThCh substrate solution; group II: same solution with incorporation of 3×10^{-5} M B.W. 284; group III: same solution with incorporation of 3×10^{-10} M DFP; group IV: BuThCh substrate solution. After incubation, the slides were rinsed, toned by ammonium sulphide, rinsed again, fixed in formalin 10 %, dehydrated in alcohol, cleared in xylol, and mounted with permount. Stained and unstained sections were observed under both ordinary light and phase-contrast microscopes.

In a series of experiments, unilateral section of three ventral roots (8th, 9th and 10th) was performed under barbiturate anaesthesia as described previously (Cerf & Chacko, 1958), and the animals were sacrificed after a 2–9 days' survival period. The spinal cord of the corresponding segments was excised and treated as above.

RESULTS

The sections belonging to groups I (AcThCh) and III (AcThCh and DFP) showed a positive enzymic reaction (Pl. 1, fig. 1), while sections of groups II (AcThCh and B.W. 284) and IV (BuThCh) remained blank (Pl. 1, fig. 2). From these results, it appears that the cholinesterase present in amphibian spinal cord belongs to the so-called specific type, or acetylcholinesterase.

The topographical distribution of the cholinesterase is illustrated in Pl. 1, fig. 1, showing a section prepared with group I solution. The pattern was essentially similar in sections of group III, although there was an indication of slight but generalized reduction in activity.

The motor nuclei exhibited a high degree of enzymic activity, often obscuring the details of individual motoneurons in the ventro-lateral part of the ventral horn. No exact intracellular localization of the enzymic activity was attempted; however, cellular characteristics were better defined when DFP was used in slightly stronger concentration, although the interneuronal spaces in the motor nuclei still remained somewhat diffusely stained. The perikaryon of motoneurons took a granular type of staining throughout, extending to varying distances into the cell processes, but the method proved inadequate to verify an eventual concentration of the enzyme at the surface membrane of neurocytes, as described by Gerebtzoff (1953), Ravin *et al.* (1953) and Koelle (1954) (Pl. 1, fig. 3). The nuclear membrane was sharply defined, but the nucleus appeared clearer in those neurons in which the details were not obscured by intense diffuse staining (Pl. 1, fig. 3). The results are in general agreement with the intraneuronal localizations reported in the microchemical study of Giacobini (1957). No other cell-bodies in the spinal cord showed such a marked cholinesterase activity: the dorsal horn cells, interneurons, and neuroglial cells were not brought out at all by the method.

The medial (grey matter) and lateral (marginal zone) neuropil areas revealed a moderate reaction best marked in a region of the dorsal horn ('dorsal neuropil') bordering the dorsal column of white matter (Pl. 1, figs. 1, 4). The staining of the lateral neuropil is evident in figs. 1, 4, 6 and 7 of Pl. 1. The possibility of its being an artifact of diffusion from the adjacent pia into the marginal zone can safely be ruled out, as no such diffusion occurred into the dorsal column from the pia bordering it (Pl. 1, figs. 1, 4). The ventro-lateral column was relatively clear and showed, if at all, only a very mild enzymic activity whereas the dorsal column appeared completely blank (Pl. 1, figs. 1, 4). The leptomeninges showed a fair degree of cholinesterase activity (Pl. 1, figs. 1, 4, 6, 7).

Blood vessels of the spinal cord were intensely stained with the AcThCh method, as seen on sections from groups I and III (Pl. 1, figs. 1 and 4–7). These vessels, of calibre ranging between 10 and 20 μ, were distributed throughout the sections. The anterior spinal artery also showed consistently a marked reaction. Thorough perfusion of the animal with saline to wash out the blood vessels revealed the localization of activity in the capillary walls.

Some of the slides yielded a transverse section through spinal roots. The ventral root revealed a cholinesterase reaction both in the axonal fibres and in the Schwann cells, but the myelin sheaths were devoid of enzymic activity (Pl. 1, fig. 6). In posterior root sections, only a very small proportion of the axons were even faintly stained histochemically (Pl. 1, fig. 7).

After unilateral ventral root section, the motoneurons showed the usual histological signs of retrograde reaction. Additionally, the motor nuclei displayed a marked diminution of the histochemical reaction on the operated side (Pl. 1, figs. 4, 5). This decrease in cholinesterase activity was not very marked 2 days after ventral root section, but became increasingly recognizable from 4-days' survival period onwards. In the ventro-lateral white column and in the lateral neuropil, no consistent alteration in depth or pattern of staining was observed on the affected side of the spinal cord; however, a reduction in staining was noticed occasionally (Pl. 1, fig. 4).

DISCUSSION

The histochemical identification of the cholinesterase present in frog and toad spinal cord with selective substrates and inhibitors shows that the enzyme belongs to the so-called specific type (AChE). This finding confirms the observations of Shen *et al.* (1955) on the frog brain. Thus, the amphibian central nervous system appears to show histochemically detectable AChE only, even in blood vessels or other non-neuronal structures which are well known to contain non-specific ChE in mammals. The phylogenetic trend in the appearance of non-specific ChE in non-nervous tissues, clearly brought out by Sippel (1955) on the heart of various vertebrates, seems to apply to the evolution of the nervous system also.

Since a histochemical method for choline-acetylase is lacking, the mapping of AChE activity has been frequently used for attempting a tentative identification of cholinergic neurons in the central nervous system. Indeed, a reasonable correlation has been observed between the distribution of acetylcholine-synthesizing regions (as evidenced by biochemical determinations of acetylcholine (ACh) content and choline-acetylase activity; see Feldberg, 1957) and that of AChE in nervous tissue. Hence, it has been suggested that cholinergic synaptic activity is exerted by neurons rich in all three components of the ACh system (Feldberg & Vogt, 1948; Burgen & Chipman, 1951; Koelle, 1951, 1954), although a few exceptions to this postulate have been reported in mammals (cerebellum, adrenergic nerve fibres). In the amphibian spinal cord, the nervous elements most obviously rich in AChE are found to be the anterior horn motor nerve cells and their processes. This observation is in agreement with the results of histochemical investigations on mammalian cord (Koelle & Friedenwald, 1949; Koelle, 1950, 1951, 1954, 1955; Gerebtzoff, 1953; Ravin *et al.* 1953; Giacobini & Holmstedt, 1958), and can be correlated with the cholinergic nature of motoneurons.

The depletion of the cellular enzyme in motoneurons after ventral root section is confirmative of observations by Hard & Peterson (1950) on the spinal cord of the dog, and can be compared to the retrograde reduction of AChE activity resulting from axonal severance in other nerve cell-bodies (Sawyer & Hollinshead, 1945; McLennan, 1954; Gerebtzoff & Vandersmissen, 1956; Brown, 1958). The mechanism of the decrease in AChE activity is not as yet properly understood since different factors could be operative. For instance, there is good evidence that other enzymic systems can be notably altered during retrograde reaction of nerve cells, and the relation of various chemical disturbances to chromatolysis has already been discussed (e.g. see Bodian, 1947). But whereas a generalized increase in activity, as observed regularly for acid phosphatase (references in Cerf & Chacko, 1958), is suggestive of enhanced enzymic function related to processes of neuronal repair, on the contrary, it seems reasonable to interpret the decreased cellular AChE activity as either a sign of motoneuron regression or lysis during intense retrograde reaction, or else as resulting from mobilization of the cellular enzyme towards a different site. Indeed, a few observations would seem to give credit to the latter view; first, an accumulation of AChE in the proximal, sprouting and regenerating segment of sectioned peripheral nerves has actually been demonstrated (Sawyer, 1946); moreover, the loss of ACh in frog's sciatic nerve following prolonged stimulation is less if the central connexions of the fibres are intact (Krause, 1955); finally, an initial increase of choline-acetylase activity was found in the central part of a sectioned sympathetic nerve (Hebb & Waites, 1956). In all these observations (see also Cavanagh, Thompson & Webster, 1954), the decrease or disappearance of ACh and its enzymes in nerve fibres severed from their cell-bodies suggests the constant passing down of the enzymes from the cells in which they originate, a view expressed by Dale (1955). Accumulation in the central end of the sectioned nerve would represent a passive piling-up by damming of the enzymes that can no longer be passed along nor be utilized. To correlate these various findings with the decreased AChE activity observed in motoneuron cell-bodies following ventral root severance, as in Hard & Peterson's (1950) and in the present study, it seems necessary to postulate further that the cellular production of the enzyme is discontinued or that an enhanced centripetal flow exists in fibres during axon reaction. No suggestions as to the possible relation of these phenomena to the reparative efforts of the neuron can be offered as yet.

No other perikarya in the spinal cord revealed demonstrable AChE activity, although electrophysiological experiments have shown that there are probably cholinergic synapses on interneurons lying in the polysynaptic reflex pathways of the cat (Feldberg, Gray & Perry, 1953). However, it is difficult to dismiss the possibility that part of the enzymic staining found in the grey matter would be localized in interneuronal processes or terminals, where a concentration of the enzyme could bring it above the method's sensitivity threshold. Indeed, in contrast to histochemical observations on mammalian tissue (see, however, Hard & Peterson, 1950), the amphibian spinal cord revealed sites of definite AChE activity in the neuropil areas of the spinal grey, particularly in a region of the dorsal horn bordering the dorsal column ('dorsal neuropil'), and in the marginal zone ('lateral neuropil'). Elements of high AChE content in the neuropil zones could be pre- or post-synaptic, or both, and the distinction is fairly difficult at the present stage. One category, at least, of post-synaptic structures belonging to cholinergic cells, viz. motoneuron dendrites, can be expected to be present in both medial and lateral neuropil areas and contribute to the AChE reaction. However, the enzymic staining at these sites does not seem to be localized exclusively in motoneuron expansions; indeed, the retrograde reaction following ventral root section does not result regularly in a decreased AChE activity in the neuropil areas, although it was shown that the axon reaction, as assessed by other methods, usually involves the whole dendritic arborizations and terminations (Cerf & Chacko, 1958). Among the various presynaptic contributions to the medial (and maybe also to the lateral) neuropil, afferent terminals from dorsal roots, and collaterals from dorsal column fibres are presumably not the site of the histochemical reaction either; it is interesting to note that the dorsal root fibres showed practically no enzyme activity, in contrast to the ventral root fibres, and that the dorsal columns appeared quite blank on the sections. From their very low ACh and choline-acetylase contents, it is generally admitted that mammalian sensory fibres are non-cholinergic (see Feldberg, 1957), although a definite but low AChE activity has been reported in dorsal roots (Koelle, 1951, 1954, 1955). In our experiments on amphibian tissue, the results of the histochemical investigation of root AChE also appear in agreement with the accepted view of the enzyme's specific role in cholinergic fibres. Other presynaptic elements contributing to the neuropil areas, i.e. axon collaterals from the white matter could be the site of part of the enzymic activity. Indeed, the sections show a mild histochemical reaction in the antero-lateral column, although part of this reaction might be localized in motoneuron dendritic processes infiltrating the tracts. Finally, even if interneuron (and dorsal horn) cell-bodies do not appear to show noticeable AChE activity, the enzyme might be concentrated in interneuronal dendritic or axonal processes participating in synaptic contacts in the neuropil zones. Thus, it is as yet very difficult to assign to definite structures the enzymic activity found in the spinal cord besides the motoneurons, and even more so to relate the histochemical findings to the nature of chemical mediation at synaptic sites; in the neuropil areas, positive identification of stained elements as axons or dendrites arising locally, or as entering fibres, must certainly await denervation studies.

The participation of neuroglial elements in the histochemical reaction is not easily assessed. The morphological characters of glial cells are not defined with the enzymic staining process as the motoneurons are. Whether neuroglial processes, which form the background framework in the amphibian spinal cord and are morphologically closely related to the neuronal elements (Silver, 1942*b*), are also involved in the histochemical reaction cannot be stated with certainty. However, the Schwann cells and their processes around the ventral root fibres do show distinct AChE activity, whereas in mammals, on the contrary, a non-specific enzyme has been detected histochemically in spinal gliocytes and in Schwann cells (Koelle, 1950, 1954). In the human brain, non-specific ChE only has been found in glial tissue by Cavanagh *et al.* (1954). As mentioned above, a phylogenetic trend in the appearance among vertebrates of the non-specific enzyme in nervous and non-nervous structures seems to apply to neuroglia. The same remark appears to hold good in the case of blood vessels which show intense AChE activity in the amphibian spinal cord, when on the contrary the predominant presence of a non-specific enzyme has been reported, for instance, in the smooth muscle fibres of blood vessels and in the capillary walls of rat brain (Koelle, 1954). Shen *et al.* (1955) also found almost exclusively AChE in frog brain capillary walls; they suggested for the enzyme a function of auxiliary 'scavenger' in the disposal of any local excess of neurally liberated ACh.

SUMMARY

Histochemical localization of cholinesterase activity in frog and in toad spinal cord, using Koelle's thiocholine technique, revealed the presence of the specific enzyme, acetylcholinesterase, exclusively. Acetylcholinesterase was found principally in perikarya of the motoneurons, and to a lesser degree in the neuropil areas of the grey matter and the perimedullary plexus. The sensory fibres in the posterior column were devoid of enzymic activity, and only a few dorsal root fibres were histochemically stained; the ventral root fibres showed a marked histochemical reaction. Schwann cells in the ventral roots revealed an acetylcholinesterase reaction. Acetylcholinesterase was also present in the walls of blood vessels in the spinal cord.

During axon reaction, the motoneuron cell-bodies showed a definite decrease in acetylcholinesterase activity.

We express our gratitude to Dr Albers and Dr Brightman of N.I.H., Bethesda, for technical advice and facilities of their laboratory where some of the experiments were carried out. We thank also Mr Parimal Ganguly of All-India Institute of Medical Sciences, New Delhi, for the photographic illustrations, and Mr F. Symons, of the University of Brussels, for preparing the plate.

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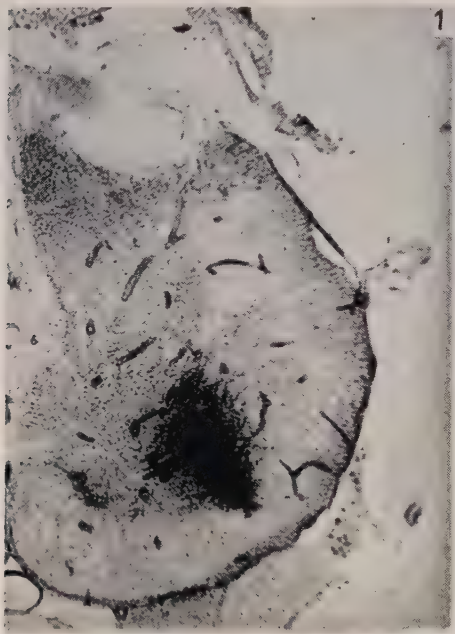

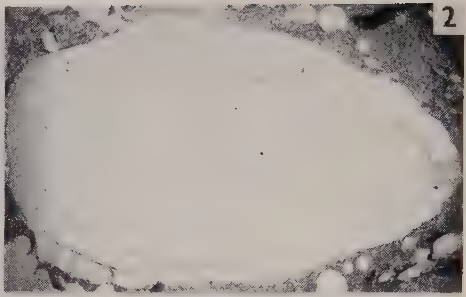

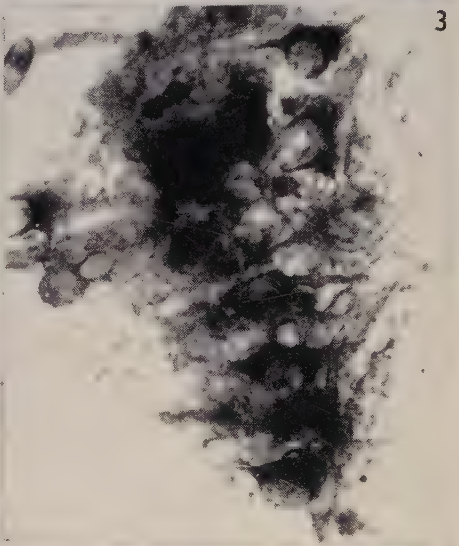

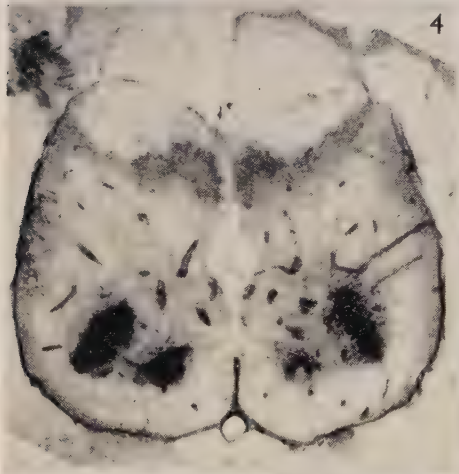

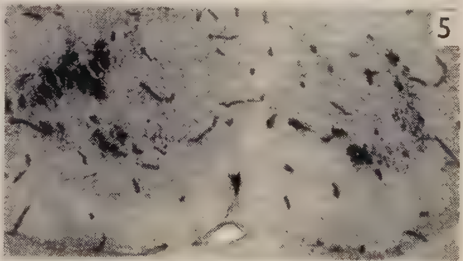

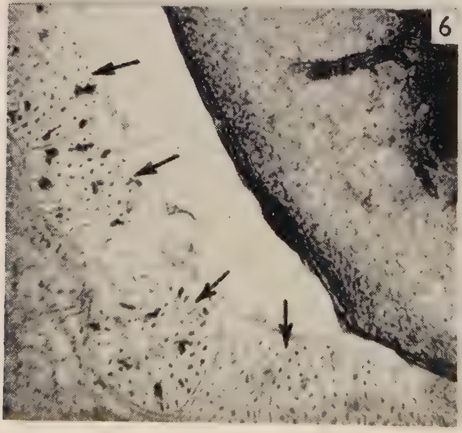

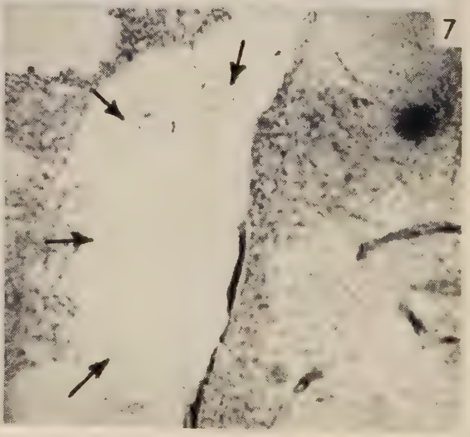

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EXPLANATION OF PLATE

All figures are photomicrographs of transverse sections through the lumbar enlargement of the toad spinal cord, some sections including spinal roots.

Fig. 1. Half section, showing a positive enzymic reaction in the motor nuclei and, to a lesser degree, in the neuropil areas. Blood vessels and leptomeninges also show a fair degree of activity. Koelle's histochemical method for the detection of acetylcholinesterase, using acetylthiocholine as substrate and no inhibitor (group I). Magnification ×47.

Fig. 2. Negative reaction throughout the spinal cord. Section incubated in acetylthiocholine substrate solution with incorporation of B.W. 284 (group II). Magnification ×19.

Fig. 3. Detail of motor nucleus, showing AChE activity extending from the cell-bodies into the proximal part of the dendritic processes. Section incubated in acetylthiocholine substrate solution with incorporation of DFP (group III). Magnification ×173.

Fig. 4. Toad in which the 8th, 9th and 10th ventral roots were sectioned on one side. Note the decreased AChE activity on the operated side (right), evident in the motor nucleus and much less marked in the marginal zone ('lateral neuropil'). Same technique as in fig. 1. Magnification ×34.

Fig. 5. Other example of unilateral retrograde reaction in the toad spinal cord, showing the decreased histochemical reaction in the motor nucleus on the operated side (right). Same technique as in fig. 1. Magnification ×45.

Fig. 6. Ventral spinal roots and part of ventro-lateral column. In the roots (pointed out by arrows), AChE activity is seen in both the axonal fibres and Schwann cells; the myelin sheaths are devoid of enzymic activity. The histochemical reaction in the marginal zone ('lateral neuropil') is particularly evident in this section. Same technique as in fig. 1. Magnification ×95.

Fig. 7. Dorsal spinal roots (pointed out by arrows) and part of lateral column. Only a very small proportion of axons show a faint histochemical reaction. Same technique as in fig. 1. Magnification ×92.

THE MEASUREMENT OF SKULL SHAPE AND SIZE

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INTRODUCTION

Cranial indices are determined by dividing one cranial measurement by another and expressing the fraction as a percentage. By taking a large number of points on the skull a great many indices have been formulated, all being expressions involving either linear or angular measurements.

Ideally, taxonomy requires perfect descriptions of structures so that good comparisons may be made and evidence of phylogenetic series obtained. It may not be possible to attain such an ideal but the attempt should be made. For general likenesses and differences verbal language may be employed to describe the appearance of structures, but when the morphology of, say, two skulls is very similar the verbal method tends to become subjective and unreliable. We turn then to mathematics since its language affords a more exact description. Today mathematical descriptions are used in form of indices, but, whilst as indices they may be perfectly accurate, they do not really describe skull shape in a systematic way. Thus, measurements are taken between many combinations of points, in many planes, and correlations may be made in many ways. However, there seems to be no simple relation between one index and another; in consequence such mathematical descriptions are complicated and may well produce confusion rather than simplification. That one could not easily reconstruct a skull from the host of indices given might indicate how lacking in order cranial measurements are, although this in itself might not be held to be proof of their inadequacy.

Indices in current use do not provide a full description of the structures measured, for the simple reason that morphological structures occupy three dimensions. Now theoretically, the shape of a skull could be expressed in the form of an equation, but the derivation of such an equation would be a formidable task. Moreover, even when obtained it would be too complex for use as a means of skull comparison. There is however a possible compromise: if significant landmarks were selected and these given a full mathematical description in space, they could then be compared with corresponding landmarks on other skulls which had been treated in the same geometrical way.

SUGGESTED METHOD OF SKULL MEASUREMENT

By means of a simple apparatus (Fig. 1), which has been devised for the purpose, the distances of selected landmarks on the skull surface from three planes are obtained. The planes used are the sagittal, the Frankfurt, and a third which passes through the external auditory meati and is at right angles to the first two. The procedure is as follows: the skull is placed in a tank of water and orientated so that the surface of the water is in alignment with the sagittal plane. The water-level is now raised until the skull is just immersed. By now lowering the water level until the sagittal plane is reached the selected landmarks in turn come to lie at the surface of the water and their distance from the sagittal plane is measured by using a ruler at the side of the tank. The same procedure is then carried out with the skull in the Frankfurt and third planes. For each skull landmark there are therefore three measurements recorded giving its distance from each of the three planes.

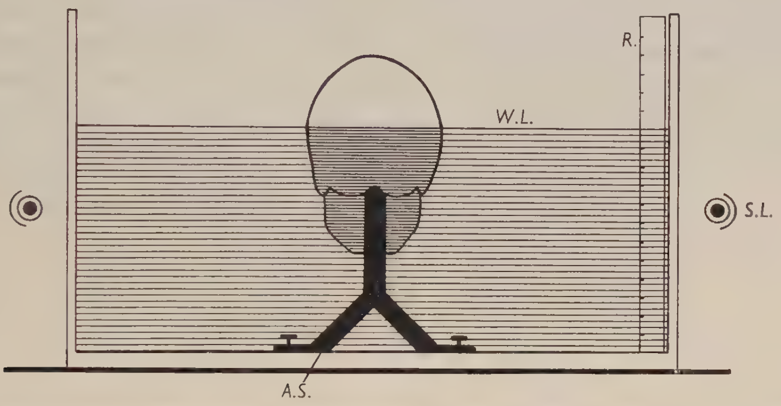

Fig. 1. Diagram of the apparatus. The skull is fixed to an adjustable stand *A.S.*, and arranged so that the Frankfurt plane is aligned with the water-level, *W.L.* As the water-level is altered and each point on the skull lies at the surface, measurements are read off the ruler *R.* *S.L.*, strip lighting.

SUGGESTED APPROACH

Since a structure has two geometrical properties, shape and size, a complete description must accurately express both. The object of the method to be suggested is to do this. It is not intended to formulate the two properties, but to afford an accurate geometrical description, in a standard way, for the purposes of comparison.

The shape of a skull may be considered as a large number of points which have a particular arrangement. If a single reference point is taken, for example a point within the skull, each landmark on the surface of the skull has its own special relation to it. The actual relation can be determined by choosing three planes which pass through the reference point and which are at right angles to one another. Cartesian co-ordinates are then used to fix the landmarks in relation to each plane and so to the reference point *R*.

In Fig. 2, x, y and z are the perpendicular distances of the landmark *P* from the sagittal, Frankfurt and third planes, the measurements having been obtained from the water-levels.

To arrive at the distance, d, which *P* lies from *R*, i.e. the modulus of the point *P* from *R*, the following formula is used:

$$d = \sqrt{(x^2+y^2+z^2)}.$$

In this paper d will be called the *Linear Index*. In this instance the landmark *P* is completely described by the expression (x, y, z), which states how far the point lies from the three respective planes. However, the expression (d) which is derived from x, y and z would only partially describe it, since while (d) states how far P is from R it does not indicate its direction or exact position.

For the purposes of comparing specimens of different sizes the expression (x, y and z) and (d) are not convenient as they are absolute measurements. Accordingly, the following indices have been devised, x/d, y/d, z/d. They are functions of the respective angles (directional cosines) which PR makes with the three planes, and may therefore be called *Angular Indices.* Since only angles are described the linear index is employed to make the description once again complete. The landmark would thus be described by the expression $(x/d,\ y/d,\ z/d){:}d$ and is then accurately located. The use of these indices, angular and linear, in a close analysis of skulls of similar type might well prove illuminating.

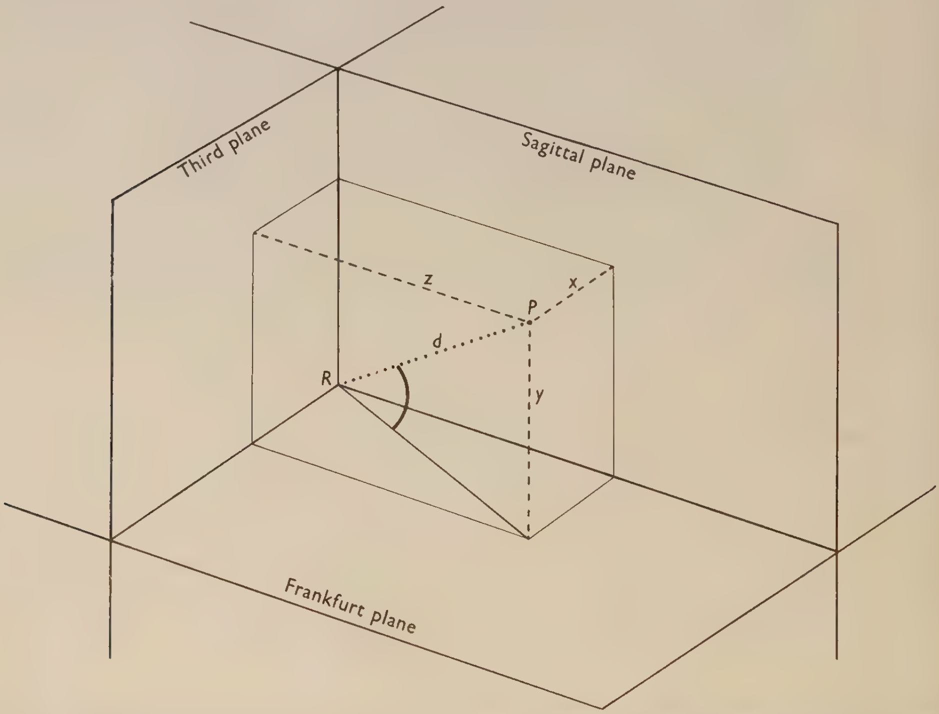

Fig. 2. x, y and z are the distances of the point P from the three respective planes. These distances are measured using the apparatus shown in Fig. 1, d is the distance of P from the homologous reference point R, and is derived from the values of x, y and z.

It must be restated that the indices are derived from the values x, y and z, which are obtained from the water-levels as previously described.

Where a point lies above the Frankfurt plane, the value of x is positive. Where it lies in front of the third plane y is positive. Conversely, points lying below the Frankfurt plane, or behind the third plane, will have respective values of x and y which are negative. For exactness and to take into account asymmetry of skulls, points lying to the left of the sagittal plane are given negative values for y, and positive if they are on the right.

DISCUSSION

There have been many approaches to the study of skull shape, each having its own advantages and disadvantages. The main problem is the complexity of cranial morphology, and the various methods used have been an attempt to simplify, so that for the purposes of comparison in ontogeny and phylogeny some simple underlying pattern might be found.

The first methods used to compare skulls were those depending upon visual observation. It enabled one to gain from the general appearance an over-all impression of skull shape and could be supplemented with more detailed descriptions of individual bones, including their relative size, shape and position. This method has been of great value in both comparative anatomy and the study of development, but is unfortunately limited where a finer analysis is required. There is a strong subjective element on the one hand, and the limitations of the eye on the other.

Methods have been adopted in an attempt to overcome these difficulties. Thus Gavan, Washburn & Lewis (1952) used photography, but were at pains to point out certain shortcomings in this technique.

With the aid of instruments such as the dioptograph outlines of the skull have been obtained and compared with others by superimposition. Benington (1911) used this technique as a method of typing skulls, and D'Arcy Thompson (1912) attempted a mathematical analysis of the measurements taken from these outlines. A method of representing skull shape by using contours was also described recently (Bunn & Turner, 1956).

It was inevitable that measurements should be used in the examination of skulls, and a host of indices has developed as various workers have added their contributions to the knowledge of cranial morphology. Such measurements and indices do not conjure up a composite picture of skull shape but indicate and measure proportion in the simplest way; nevertheless, the method has been of immense value, reducing as it does certain subjective errors, and also because it offers a more exact way of making comparisons by measuring the degree of similarity or difference.

Methods of analysing the results of craniometry have evolved to the point where a specialized knowledge of mathematical manipulation is required, and for the anatomist who depends upon what he sees or feels, or on what he can measure, many of his observations become lost in the complexity of mathematical analysis.

The method described in this paper is designed to extract the maximum amount of geometrical information from skull measurements and to express these in the most compact form. The measurements can then be used for any mathematical treatment considered necessary.

The principle used is based on the simple system of Cartesian co-ordinates, and individual points are related one with another by means of a single common reference point. This point lies towards the middle of the skull, and for practical purposes is located by means of planes which can be recognized on the outer surface of the skull.

The reference point R is defined as the point of intersection of three planes which lie at right angles to one another. They are the sagittal, Frankfurt and a third plane (which is defined as the plane which lies at right angles to the other two and passes through the external auditory meatus). The three planes are homologous in skulls of animals belonging to different species, and similarly the reference point will be homologous. For this reason the name *homologous reference point* has been chosen.

The more points of the skull described, the better the comparison made. Limitation as to number will depend mainly on being able to agree upon significant landmarks.

There is a minimum number of indices which can be used to give a *complete description*. In the suggested method eighty indices (i.e. 20 sets of 4) are necessary to describe fully twenty landmarks. This is the smallest number for *any* method. With reference to current methods, 17,955 possible indices could be formulated involving these twenty points; and it would prove impossible actually to reconstruct the shape of a skull given any number of these. The only resort is to consider a manageable large number of indices for making a comparison. This would amount to only a minute (almost negligible) sample. This state of randomness, due primarily to a lack of system, is clearly an unsatisfactory method for comparing skulls, particularly closely similar ones, since there is no chance of obtaining a complete description. The crucial points of using indices for comparison are: (1) Do the indices used give us *all* the information *available*? (2) If so, do they carry this information efficiently, i.e. is the maximum amount of information available carried by the minimum number of indices? By satisfying the first question it is possible to make the best possible comparison, and by satisfying the second question the comparison itself is made easier. The method suggested does satisfy both requirements.

Closely related to the problem of sampling is the importance which may be attached to certain landmarks. If, for example, the nasion is used in the determination of *ten* indices, whilst the inion is used for only *two*, then the former has been given an exaggerated importance from the point of view of comparison. If it is agreed that all points should be considered to be of equal importance when shape is being determined, then each should be given equal weight when shape is being compared.

Since the suggested method affords a description of *single points only*, every point can be given its full significance when comparisons are made.

From a table of these angular and linear indices it would be possible to reconstruct the skull accurately. The inefficiency and randomness of the current methods would make such a reconstruction impossible.

SUGGESTED APPLICATIONS OF THE METHOD

The use of the method in ontogenetic studies seems possible. For example an investigation of the relative growth rates of the cranial vault and facial skeleton might profitably be made in this way, and indeed, provided three planes could be established, its use might be extended to the study of differential growth in any part of the body.

In phylogenetic studies, too, the use of homologous reference points, obtained from the intersection of three agreed planes, would enable valuable comparisons to be made between different animals whether living or fossil.

It seems, too, that for a comparison of growth changes in different animals these indices could be of value in enabling the positional changes of landmarks occurring with age to be followed.

SUMMARY

1. A system of cranial indices has been devised, based on the geometrical description of points. The description is complete from the mathematical point of view and compared with the current method more efficient.

2. Cranial landmarks are related to one another in space through the medium of the reference point, which is determined by the intersection of three fixed planes perpendicular to one another.

3. As a basis for comparison, a homologous reference point fixed by homologous planes in different skulls has been suggested, although another reference point can be taken if desired.

4. The system of indices used for each point as related to the reference point are: $(x/d, y/d, z/d):d$. These are called angular and linear indices and they afford a complete geometrical description in space.

5. Possible applications of the method have been suggested.

We should like to thank Prof. E. W. Walls for his great interest and encouragement in the preparation and writing of this paper, and Miss E. D. Hewland for preparing the figures.

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THE ARTERIAL SUPPLY AND VENOUS DRAINAGE OF THE VERTEBRAL COLUMN OF THE DOG

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In recent years orthopaedic surgeons have become increasingly aware of the importance of comparative anatomy and pathology. Because of this renewed interest, the dog is being used more frequently for experimental work on the vertebral column and hip joints by surgeons and veterinarians alike. It would seem timely, therefore, to reassess the work on the blood supply of the dog's vertebrae.

Apart from a general anatomical description of the arteries supplying the spine, the standard reference works provide inadequate detail on the intraosseous distribution of the vessels (Miller, 1948; Bradley & Grahame, 1959).

The interest aroused in the vertebral venous system in man by the work of Batson (1940) has been extended to the dog by Worthman (1956). His papers on the anatomy and functional aspects of the longitudinal vertebral venous sinuses of the dog do not, however, contain a full account of the arrangement of veins within the vertebrae.

This paper is designed primarily to describe the anatomy of the arteries and veins as they are found within the vertebrae of the dog.

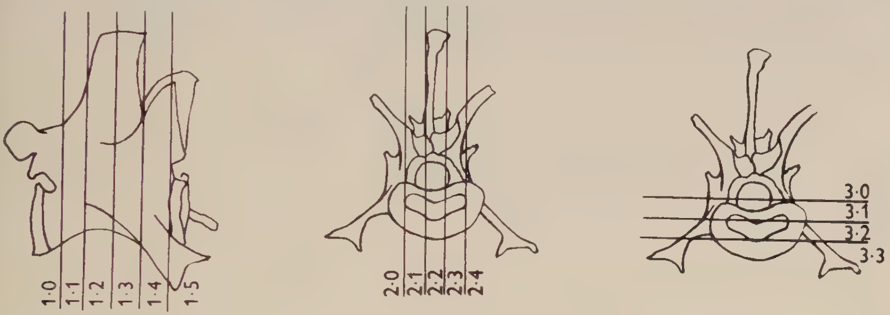
MATERIALS AND METHODS

Specimens were obtained from the cervical, thoracic and lumbar vertebrae of fifteen normal dogs. The arterial supply to the vertebral column was studied in ten dogs, and the venous drainage in five. These animals varied in age from 8 weeks to 12 years. The group was made up of different breeds, and included two adult cocker spaniels, four greyhound racing dogs, one bull terrier cross breed and eight mongrel dogs. They were all killed with intravenous doses of pentothal sodium, and injected with a suspension of barium sulphate (Micropaque), alone or mixed with 10 % Berlin Blue, within a few minutes of death, by the following technique:

The thorax was opened between the fourth and fifth ribs on the left side. A polythene cannula was then inserted in a caudal direction into the thoracic aorta and tied in place. In addition, a cannula was also placed in the common carotid artery on one side of the neck, directed cranially. For the venous specimens, a similar cannula was inserted into the caudal vena cava from the right side of the chest or passed upwards into it from the femoral vein.

The injection medium was then delivered through the cannula under constant pressure (7–10 lb./sq. in. arterial, 5–7 lb./sq. in. venous), from an oxygen cylinder source. The apparatus used was of simple construction, as recommended by Tompsett (1957). The volume of Micropaque injected varied according to the size of the animal. It is extremely difficult to obtain uniformly good filling of the intraosseous veins of the vertebrae. The large clots which appear in the caudal vena cava soon after death are broken up into small fragments by the barium injection mass.

Consequently, the veins in many specimens may become obstructed and they fill only partly with the barium suspension. In order to overcome these difficulties a large dose of anticoagulant (Pularin Heparin) was administered to one dog while under anaesthesia, and this was allowed to circulate for a few minutes before the lethal dose of pentothal was administered. The injection of the vertebral veins obtained in this case was of a high standard. Notwithstanding the difficulty produced by clot fragmentation, good specimens may be obtained in most cases if two conditions are observed while making the injection. The first is to ensure flow of the medium under a constant adequate pressure, and the second, to allow the fluid to flow into the body until its delivery comes to a halt.



Text-fig. 1. Key A, Key B, Key C. (For explanation see text.)

After the injection of Micropaque, the vertebral columns were removed and fixed in formol saline for at least 5 days. Then they were decalcified in 15 % nitric acid, or 10 % formic acid if histological sections were to be made. The vertebrae were then divided into slices, longitudinal, horizontal and transverse sections being made. These were radiographed, using a water-cooled low voltage medical X-ray unit with a beryllium window and a fine grain emulsion film. Some of the specimens were subsequently cleared by the Spalteholz technique, and examined under the dissecting microscope. In this way it was possible to supplement the information obtained from the radiographs which were otherwise difficult to interpret.

In the legend accompanying the illustrations made from the radiographs of these specimens, each figure is followed by a key number. The key number, taken from the three line drawings reproduced in this paper (Text-fig. 1), shows the precise origin of the section of the vertebra which has been radiographed.

THE ARTERIAL SUPPLY

The lumbar spine of the dog is the most readily accessible area for experimental work on vertebral bodies. Because it is used most frequently for this purpose the salient features of the gross anatomy of the lumbar arteries will be described. That aspect of the thoracic and cervical vertebral blood supply will be dealt with more briefly, but equal attention will be focused on the intraosseous distribution of arteries in these three areas of the spine.

THE GROSS ANATOMY OF THE LUMBAR ARTERIES

The lumbar vertebrae are supplied by seven pairs of vessels arising from the dorsal aspect of the thoracic and abdominal aorta and from the internal iliac arteries.

The first pair are quite small and arise from the thoracic aorta above the diaphragm.

The second pair are larger and arise from the abdominal aorta between the crura of the diaphragm at the level of the disc between the first and second lumbar vertebrae.

The third pair of vessels arise at the level of the disc between the second and third lumbar vertebrae, at the same level as the site of origin of the right renal artery from the aorta (Pl. 1, fig. 1).

The fourth pair of lumbar arteries arise at the level of the disc between the third and fourth lumbar vertebrae, and they pass laterally over the attachments of the long tendons of the diaphragm to proceed obliquely across the body of the fourth lumbar vertebra.

The fifth pair arise just below the fourth at the level of the middle of the fourth vertebral body. The vessels proceed downwards in the mid-line of the spinal column, running parallel to each other, wedged between the psoas muscles (Pl. 2, fig. 2). After crossing the disc between the fourth and fifth lumbar vertebrae, they run across the body of the fifth lumbar vertebra.

The sixth pair of vessels arise at the level of the bifurcation of the aorta, over the middle of the body of the fifth lumbar vertebra. These are slender vessels and they diverge slowly from each other at the level of the disc between the fifth and sixth lumbar vertebrae to cross obliquely the body of the sixth lumbar vertebra.

The seventh pair are the most slender of all the lumbar arteries. They arise at the level of the middle of the sixth vertebral body, in common with the origin of the median sacral artery, or from the hypogastric arteries (Bradley & Grahame, 1959).

Each lumbar artery, therefore, eventually passes obliquely across its corresponding vertebral body, being crossed laterally by the sympathetic trunk. The main trunk proceeds towards the caudal and medial part of the intertransverse aponeurosis, which it pierces. Between its origin from the aorta and its passage through this aponeurosis, the vessel gives off a series of small branches. One branch enters the bone where a basivertebral vein emerges on the abdominal surface; this supplies the centrum. Others penetrate the ventro-lateral aspects of the vertebral body cranially and caudally to supply the epiphyses (Pl. 2, fig. 2). In addition, a number of slender branching vessels are given off and these enter the deep surface of the psoas muscle.

Where the main lumbar artery penetrates the intertransverse aponeurosis, it is lying on the dorso-lateral aspect of the intervertebral disc. It divides there into two main branches. One passes laterally along the transverse process, and from it large branching vessels pass into the sacrospinalis muscle mass laterally. The other crosses the intervertebral foramen, passing dorsal to the emerging nerve root, and it proceeds caudally and backwards in close relation to the mamillary process and the lateral side of the zygapophyseal joint of the caudal vertebra. It winds around this structure to pass on to the lamina, and thence medially and farther backwards to ts termination in fine branches along the side of the spinous process of this adjacent caudal vertebra. It is from this major branch of the lumbar artery, in the region of the intervertebral foramen, that a number of fine branches arises which will supply the posterior aspects of the vertebral bodies. In its course across the dorsal elements of the vertebrae, many branches are given off to supply the bulk of the sacrospinalis muscles and many small vessels pass into the bones. Consequently each lumbar artery is seen to supply branches not only to its corresponding vertebral body on its ventral aspect, but to the neural elements of its own and those of the vertebra immediately caudal to it after its passage through the intertransverse aponeurosis.

THE INTERCOSTAL ARTERIES

The intercostal arteries give off branches to the centrum and epiphyseal areas in the same way as do the lumbar arteries, between their origin from the aorta and their point of division at the intervertebral foramen.

THE VERTEBRAL ARTERIES

In the cervical spine the vertebral arteries give off small ventral branches at each intervertebral foramen. These curve around the ventro-lateral sides of the vertebral bodies, giving off extraspinal epiphyseal arteries. The main trunks of these fine arteries then converge towards the centre of the vertebral body, breaking up into a multitude of branches, some of which enter the vertebra, while the remainder enter the overlying ventral cervical muscles (Pl. 2, fig. 3).

Using standard methods of dissection, it is possible to demonstrate only a few of the vessels which are to supply the vertebral body, after the arteries have been injected with latex rubber (Pl. 2, fig. 2). They are all, without exception, very fine branches. Using the barium suspension, Micropaque, the vessel patterns within the vertebrae can be demonstrated clearly after injection, in decalcified sections submitted to X-ray examination.

THE ARTERIES OF THE VERTEBRAL BODY

The arteries supplying the body of the growing vertebra are distributed in a similar manner to those seen in a growing long bone (Morgan, 1959), with the difference that more than one nutrient artery always enters the centrum (Pl. 2, fig. 4). Likewise, while the epiphyseal plates remain open there is no anastomosis between metaphyseal loops and the arteries of the epiphyses (Pl. 2, fig. 5, 6).

THE EPIPHYSEAL BRANCHES

The vertebral bodies are penetrated by arteries on their spinal and ventro-lateral aspects. The origins of the extraspinal epiphyseal arteries have been described above (Pl. 2, fig. 2)

These vessels penetrate the ventro-lateral aspects of the vertebral body. They course centrally in the transverse plane, breaking up into many small branches as they proceed (Pl. 2, fig. 7). The contributing branches of arteries supplying the epiphysis from its spinal and extraspinal surfaces anastomose with each other (Pl. 2, fig. 8).

The intraspinal epiphyseal arteries spring directly from the arterial arcades found on the dorsal surfaces of the vertebral bodies (Pl. 2, fig. 9). These arcades will be described in more detail below.

Within the growing epiphysis there is a complex pattern of distribution of the main arteries. Adjacent to the disc surface, beneath the thin cartilage end-plate of the vertebra, there is a densely woven arteriolar network (Pl. 2, fig. 5). On the growth plate side of the epiphysis, there are numerous fine arteriolar branches given off from the parent stems of the epiphyseal arteries. However, the concentration of arterial loops over this area is much lower than that beneath the end plate or at the metaphyseal side of the epiphyseal plate (Pl. 2, fig. 5).

When the growth plates of the vertebral body close, the concentration of arterial loops in the metaphyseal zone falls and loosely knit anastomoses are established between the arteries of the two regions (Pl. 2, fig. 10).

THE ARTERIES OF THE CENTRUM

(*a*) *Extra-spinal branches*

The pattern of distribution of the arterial supply to the centrum is essentially the same in typical vertebrae from the lumbar, thoracic and cervical regions of the spine in dogs of all ages and of differing breeds. Assessed on the calibre of the injected vessels, the main contribution appears to come from arteries entering the ventral surface of the vertebrae, in most cases (Pl. 2, figs. 6, 10; Pl. 3, fig. 11), but occasionally the main vessel enters the centrum from behind (Pl. 3, fig. 12). Irrespective of the site of origin of this main parent trunk, once it has entered the centrum it breaks up into a tree-like pattern, its branches spreading out in all directions. In longitudinal sections, these vessels can be seen passing out towards the metaphyseal ends of the vertebral bodies (Pl. 2, fig. 10). The three-dimensional perspective of this arterial pattern is completed by examining horizontal and transverse sections taken through the centre of vertebral bodies (Pl. 2, fig. 4; Pl. 3, fig. 13). It will be noted that the main arterial supply to the centrum runs into the vertebra along the basivertebral canals (Pl. 3, fig. 11).

(*b*) *Intraspinal branches*

At the level of the intervertebral foramina, the lumbar and intercostal arteries and the vertebral arteries in the cervical spine, all give off a small branch which enters the spinal canal and further subdivides. One branch breaks up to supply the nerve roots and spinal cord.

Another passes dorsally, dividing into a variable number of branches, which enter the pedicles and laminae, being distributed within the bone to the various elements which make up the neural arch (Pl. 3, figs. 14, 15). A small main vessel passes into each separate element and runs a straight course towards its termination. For example, a main stem artery runs from the centre of the pedicle across the lamina and up into the corresponding zygapophyseal process. Small branches are given off from each side of it along its whole course (Pl. 3, fig. 14).

The spinous process contains a similar vessel running centrally within it. Each of these arterial branches is closely related to a venous channel (Pl. 3, fig. 16).

The anastomoses within the bone, between these vessels from within the spinal canal and those from outside it are shown in Pl. 2, fig. 4.

The third of these main branches entering the spinal canal at the intervertebral foramen courses on to the dorsal surface of the vertebral body, and joining with branches from foramina above and below, helps to form arterial arcades on each side. These vessels are arranged in arcs converging on the centre of the vertebral body from each side. They mirror the pattern of the longitudinal vertebral venous sinuses which actually overlie them (Pl. 2, fig. 9; Pl. 3, fig. 17). From these arcs, branches pass into the transverse processes and the bases of the pedicles laterally, into the epiphyseal zones and via the basivertebral canals, into the centres of the bodies of the vertebrae. Delicate branches spring from the convexities of adjacent arcs in the regions of the intervertebral discs. These contribute to the rich anastomosis between the intraspinal arteries which supply the vertebral bodies. Fine irregularly arranged branches also form a network around the longitudinal vertebral venous sinuses. These minute vessels are destined to supply the soft tissues around the spinal theca. They will not be considered in any further detail.

With advancing age, the contribution of the intraspinal arteries to the blood supply of the vertebral body in the dog appears to diminish. The calibre of these vessels is certainly very small compared with the size of those which enter from the ventral aspects of the vertebral bodies. This difference is seen when Pl. 2, fig. 10, and Pl. 3, fig. 11, are compared.

THE VENOUS DRAINAGE

(*a*) *The longitudinal vertebral venous sinuses*

The venous drainage of the dog's vertebra centres around the longitudinal vertebral venous sinuses, which lie on the dorsal aspects of the vertebral bodies. These are vessels of large calibre (2–4 mm. in diameter), arranged segmentally in arcs, the right and left convexities of which approach each other in the mid-line of each vertebral segment (Pl. 3, fig. 17). Worthman (1956) has described their extravertebral connexions in considerable detail. The present study confirms the findings recorded in his papers, but provides more information on the intraosseous distribution of the vertebral veins.

At each intervertebral foramen, where one arch joins the next in the arcade, intervertebral veins connect the sinus of the side with veins outside the vertebral canal (Pl. 3, figs. 17, 18). The course taken by veins draining the spinal cord and theca will not be considered in this paper.

Worthman (1956) stated that there were connexions between the convexities of the right and left sinuses in the middle of each vertebra, in close relation to the dorsal longitudinal ligament. Basivertebral veins make connexions between these anastomoses and extravertebral veins on the ventral surfaces of the vertebrae.

(*b*) *Basivertebral veins*

In the present series of vertebrae, the basivertebral veins frequently joined the dorsal longitudinal sinuses directly. However, deeper within the vertebral bodies, the basivertebral veins, if they were paired, always anastomosed with each other (Pl. 3, fig. 19; Pl. 4, fig. 20). In the case of the single basivertebral vein, Worthman's description always applied, with the exception that the single basivertebral vein sometimes divided into two branches just before emerging from the ventral aspect of the vertebral body (Pl. 4, fig. 21).

(*c*) *The veins of the centrum*

The tributaries of the basivertebral vein within the centrum were arranged predominantly at right angles to its main axis. This is clearly shown in longitudinal sections (Pl. 4, figs. 22, 23). The arrangements are shown to be somewhat more complex than this when specimens are viewed in horizontal sections (Pl. 4, figs. 24, 25). It is apparent then, that the basivertebral vein is the centre of a rosette, but its largest branches are still directed in the long axis of the vertebra. These branches appear to originate within a short distance of the metaphyseal zones of the vertebra, from whence they course centrally towards the basivertebral vein or veins. It is evident, therefore, that the venous drainage of the centrum follows a regular basic pattern, but it is subject to minor variations, depending on the arrangements of the basivertebral veins (Pl. 3, fig. 19; Pl. 4, figs. 20, 21).

(*d*) *The epiphyseal veins*

There was one constant feature noted in vertebrae from any region of the vertebral column, both in growing and adult dogs, of different breeds. This was the striking pattern of the veins draining the epiphyseal zones of the vertebrae. Fine arborizing veins, which were bilaterally symmetrical, drained by slender stems directly into the longitudinal vertebral venous sinuses. These veins appeared to have no connexion with extravertebral veins (Pl. 3, fig. 18, Pl. 4, figs. 22, 26). This same basic pattern was seen in the vertebrae of an 8-week-old dog examined in this series. The accompanying illustration shows the outline of the main epiphyseal veins, behind which the brush-like pattern of the veins draining the centrum is seen (Pl. 4, fig. 27). In contrast to the findings in the adult dogs, many fine veins are arranged radially around the outside of the epiphysis, draining directly into extravertebral veins.

(*e*) *The neural element veins*

The various features of the venous drainage of the neural elements of the vertebrae and of the transverse processes are illustrated in Pl. 3, fig. 18; Pl. 4, figs. 21, 22, 24 and 28. The spinous processes are drained by a large vein which traverses the length of the process, lying within the bone adjacent to its cranial edge. It is intimately related to the accompanying artery (Pl. 3, fig. 16; Pl. 5, fig. 29). The tributaries which join this main channel at varying levels along its length are connected across the periosteum with extravertebral veins in the surrounding soft tissues.

On reaching the lamina this central spinous vein bifurcates (Pl. 4, fig. 21, 28), crosses the lamina and enters the pedicle. In the pedicle, veins from the transverse processes and from the neural elements finally come together. A short pedicle vein is thus formed, running parallel to the dorsal surface of the vertebral body in the base of the pedicle. It finally emerges from each end of the pedicle at the intervertebral foramina bounded by it, to join the confluence of intraspinal and extraspinal veins.

In addition, a few small venous radicles emerge from the spinal surfaces of the laminae and drain into veins around the spinal theca. These are inconstant.

In the vertebrae of the 8-week-old puppy examined, there was another significant deviation from the findings on the venous drainage of the vertebrae in the adult dogs; that concerned the tributaries of the larger calibre vessels such as the basivertebral veins. These tributaties were of extremely fine calibre and their concentration in a given area was high. In the radiographs, the outlines of the main vessels are shown lying in a more or less homogeneous grey background, made up of these numerous delicate venous channels. The orientation of these tributaries is identical with the much larger analogues in the adult. It will be noted in Pl. 5, fig. 30, for example, that the centrum veins course towards the basivertebral vein and enter it at right angles.

DISCUSSION

During the preparatory planning of an experimental investigation on the dog's vertebral column it was found that the detailed information on the anatomy of the arterial supply and venous drainage of the spine was deficient. Standard veterinary anatomical texts provided descriptions only of the larger vessels related to vertebrae. Likewise, a thorough search of the literature published on the anatomy of the dog confirmed the fact that there was a hiatus in the knowledge of intraosseous vessel patterns in vertebrae.

This study was designed to fill the hiatus so that the details it provided, taken with information already available, would permit one to have an integrated and more complete knowledge of this anatomy.

Certain points have emerged from this work which have a potential practical importance for those interested in experimental surgery on the dog's vertebral column.

Definitive patterns of the arterial supply and venous drainage of the dog's vertebrae were present soon after birth. During the period of growth there were only two significant differences in intraosseous vessel patterns from the findings in adult dogs. The first concerned the arterial arrangements in relation to the epiphyses of the vertebral bodies. Attention was drawn to the complete absence of anastomoses between epiphyseal and metaphyseal vessels in the vertebral bodies of growing dogs. In recent years, following the work of Rubaschewa & Priwes (1932) and Trueta (1957), such findings have come to be recognized as the normal arterial patterns adjacent to all growth plates. The same findings have been recorded in the vertebral bodies of growing rabbits (Amato & Bombelli, 1959).

The second point of difference between the young and adult dogs concerned the over-all concentration of fine branches of both arteries and veins. Although the basic definitive patterns of main branches were present in very young dogs and remained constant throughout adult life, the concentration of fine branches of the arteries and of the tributaries of major veins was far higher in the young dogs. Throughout the growth period there appeared to be a gradual decrease in the number of branches of the vascular trees until the final adult patterns emerged after fusion of the epiphyses of the vertebral bodies.

For reasons already mentioned in this paper it would seem important to draw particular attention to the distribution of the lumbar arteries. Although these vessels are of large calibre only small branches pass from them into the lumbar vertebrae. This is almost certainly true in the case of the human lumbar vertebrae too, yet the possibility that such is the case does not appear to be widely appreciated (Prignacchi, 1956).

In the dog, it appears that the neural elements of a particular lumbar vertebra receive their arterial supply from branches of two lumbar arteries on each side. The blood supply of the vertebral body contrasts markedly with this finding. The ventral aspect of an individual lumbar vertebra is penetrated by a small series of branches derived solely from the two lumbar arteries which cross it. The dorsal surface of the vertebral body is supplied with branches derived from an arterial arcade with rather extensive cranial and caudal anastomoses. Despite these anastomoses, which have been illustrated in this paper (Pl. 2, fig. 9) the calibres of the branches entering the dorsal aspects of the vertebral bodies have been shown to be smaller than those of corresponding branches which enter from the ventral surface of the vertebral body. This applies only to the adult dog. It may well be, therefore, that in the adult dog the neural elements of the lumbar vertebrae have a richer arterial supply than the vertebral bodies. This finding has been of great interest to us because of its possible implications in relation to the study of spinal fusion techniques in the dog.

Finally, the arrangements of the epiphyseal veins have been studied in considerable detail in this paper. We have been unable to find any published work which shows similar radiographs of these veins.

SUMMARY

1. The intraosseous blood supply of typical cervical, thoracic and lumbar vertebrae from dogs has been studied. Specimens were obtained from fifteen animals of varying ages and different breeds.

2. The techniques of radiography and clearing of some specimens (Spalteholz), following the injection of a barium sulphate suspension (Micropaque) alone or mixed with 10% Berlin Blue, have provided detailed information of the vessel patterns within the vertebrae.

3. Findings recorded supplement the available published accounts of the anatomy of the arterial supply and venous drainage of the dog's vertebrae.

I wish to thank Prof. J. Trueta for granting me the facilities to carry out this work and for his constant encouragement. I am indebted to Prof. E. C. Amoroso, F.R.S., for the most useful suggestions he made during the preparation of this paper.

My veterinary colleagues, Mr L. C. Vaughan of the University of London and Mr Heather of Oxford, gave me invaluable assistance in obtaining the animals used in this study. Mr D. W. Charles helped at every stage in the preparation of the specimens and produced the illustrations. Miss M. Litchfield prepared the histological sections. I am most grateful to all of them.

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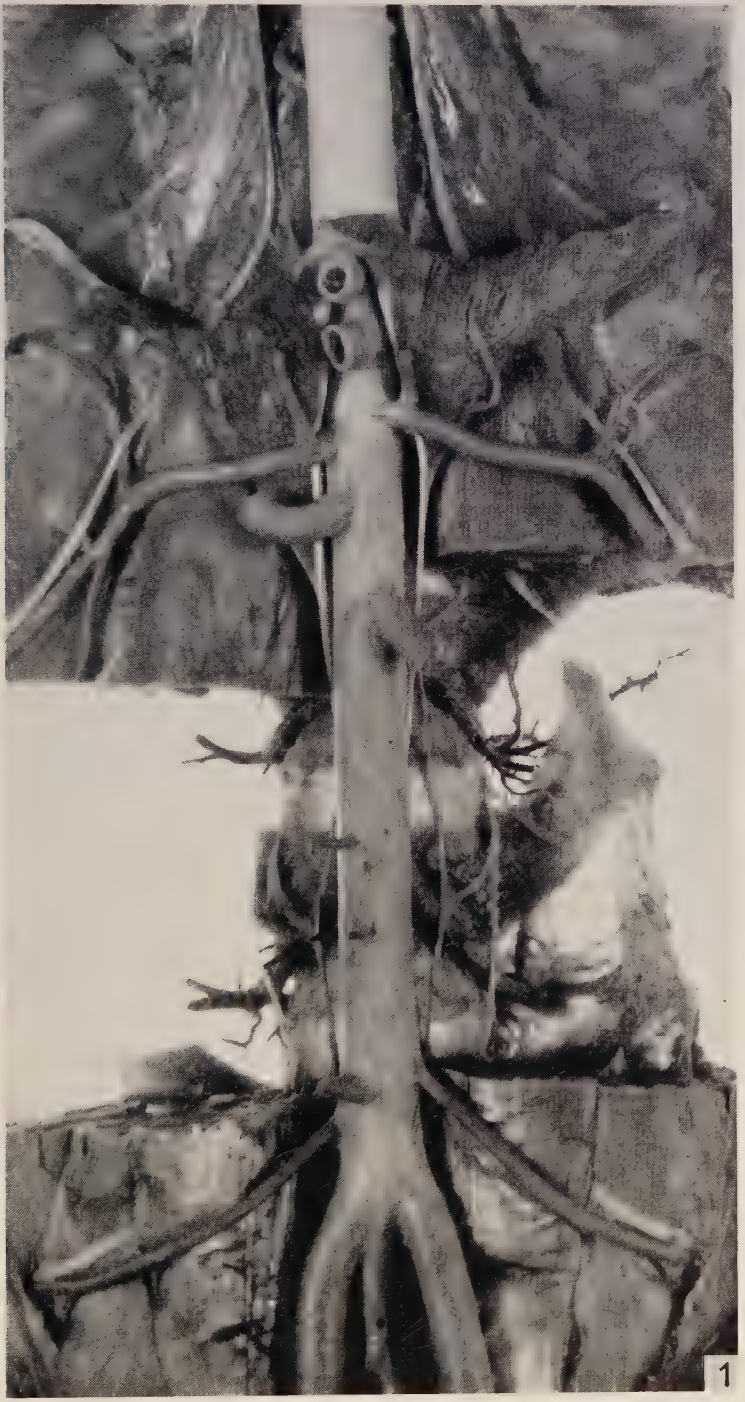

CROCK—THE ARTERIAL SUPPLY AND VENOUS DRAINAGE OF THE VERTEBRAL COLUMN OF THE DOG

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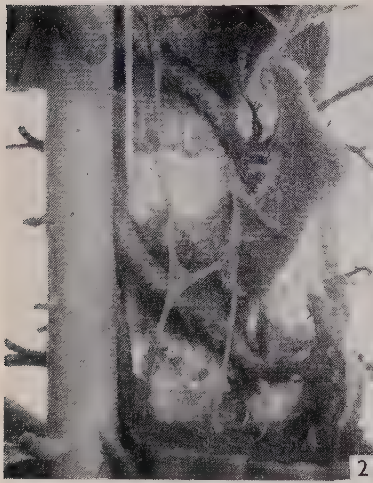
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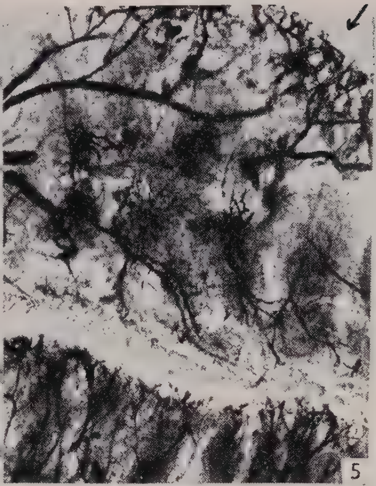
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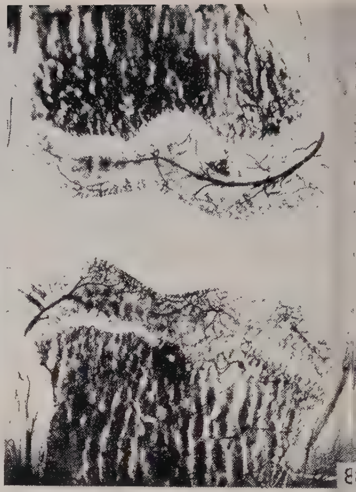
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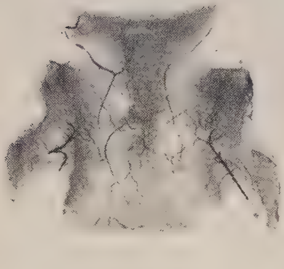
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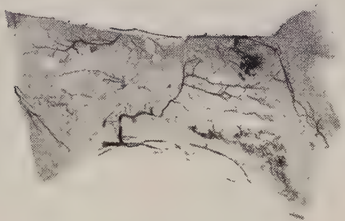
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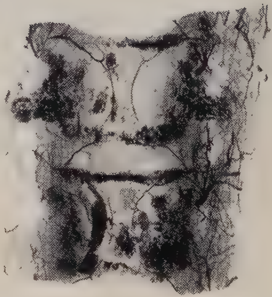
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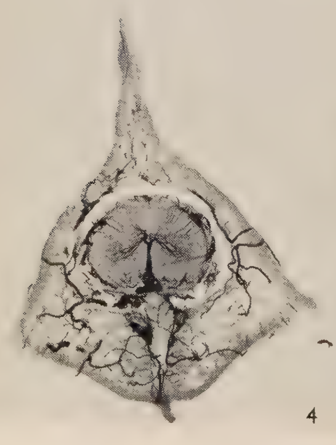
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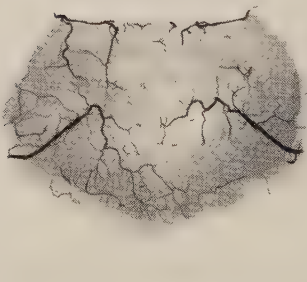
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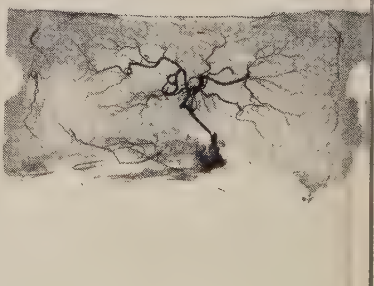
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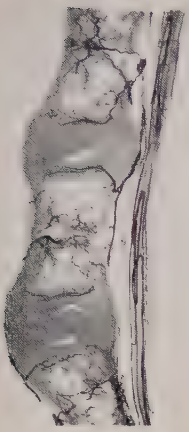
CROCK—THE ARTERIAL SUPPLY AND VENOUS DRAINAGE OF THE VERTEBRAL COLUMN OF THE DOG



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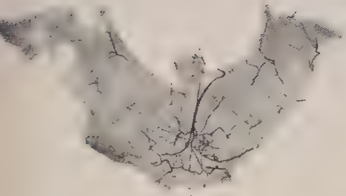

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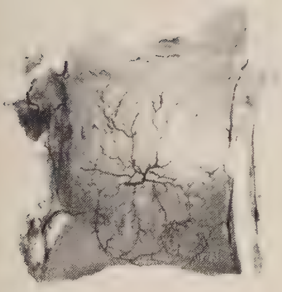

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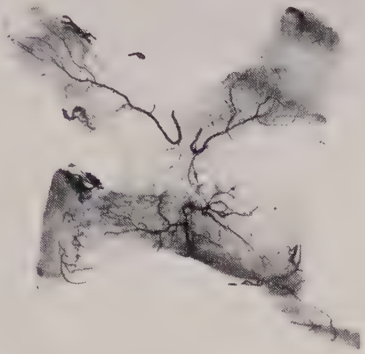

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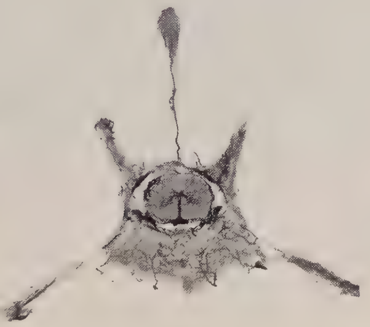

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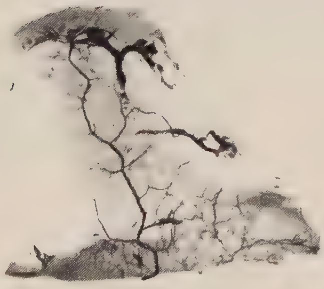

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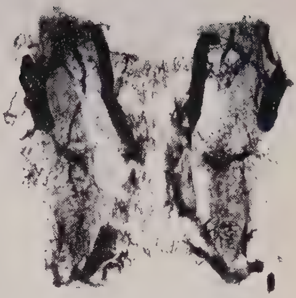

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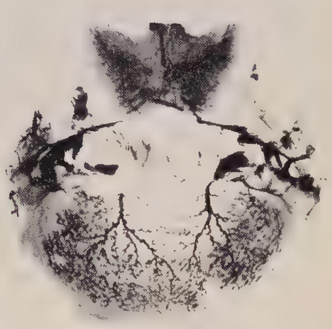

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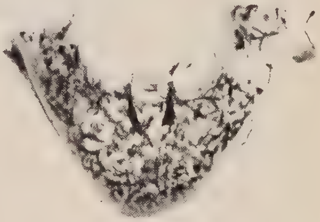

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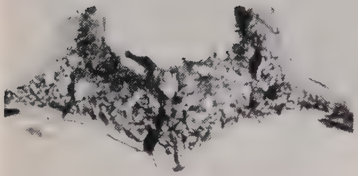
CROCK—The arterial supply and venous drainage of the vertebral column of the dog



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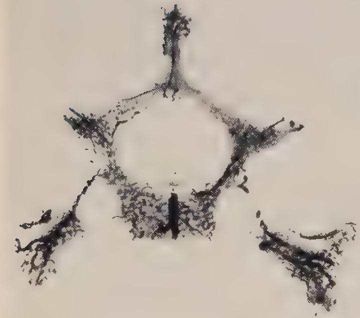

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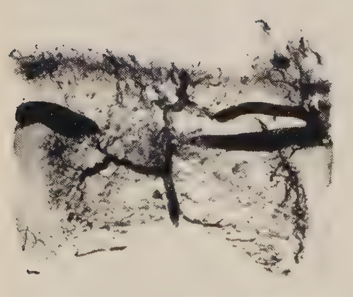

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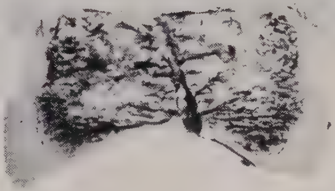

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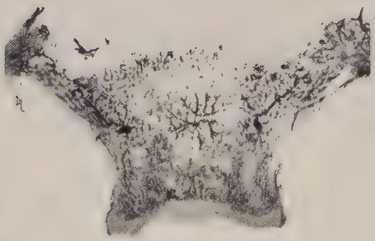

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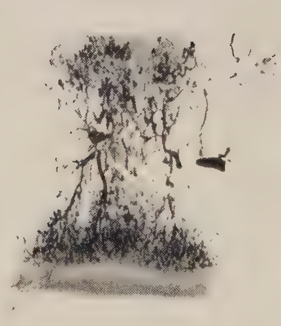

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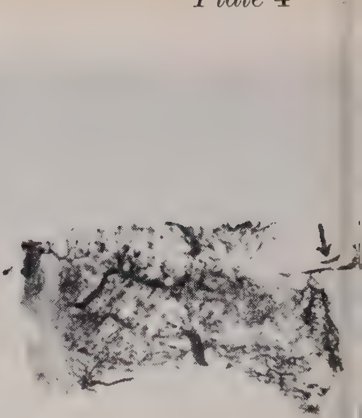

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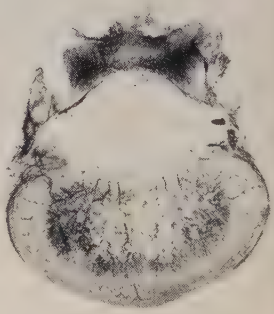

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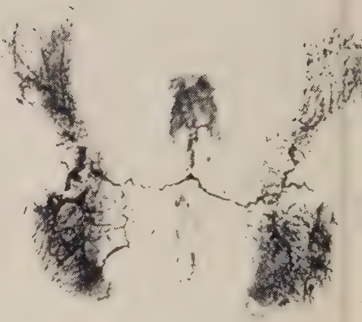

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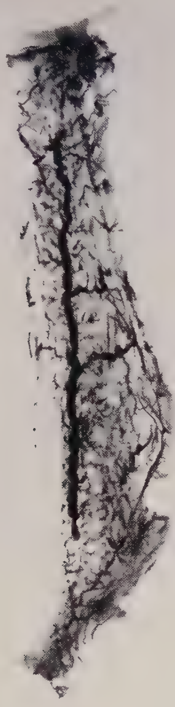
CROCK—THE ARTERIAL SUPPLY AND VENOUS DRAINAGE OF THE VERTEBRAL COLUMN OF THE DOG



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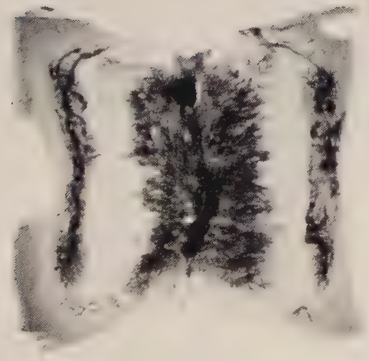

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CROCK—The arterial supply and venous drainage of the vertebral column of the dog

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A dissection of the dorsal abdominal wall. Greyhound, aged 4 years. The aorta and its branches have been injected with red latex rubber before dissection. The relationship of the aorta and some of its main branches to the lumbar spine is shown. On the left side of the specimen above the diaphragm, one intercostal artery is seen. Lower down, the transverse process of the fourth lumbar vertebra has been cut away.

PLATE 2

Fig. 2. A detailed oblique view of the specimen illustrated in Pl. 1, fig. 1. The tendon of the diaphragm is seen attached to a tubercle on the upper border of L.V. 4. The third, fourth and fifth lumbar arteries are shown. Note the two small branches arising from the lower border of the fourth lumbar artery. These vessels penetrated the vertebral body just cranial to the disc shown, to enter the epiphyseal zone. These are referred to in the text as extraspinal epiphyseal arteries. This fourth lumbar artery is shown penetrating the intertransverse aponeurosis. The fifth lumbar artery is seen arising opposite the middle of the body of the fourth vertebra. It is running parallel to the aorta.

Fig. 3. (Key C 3.3.) Cervical vertebral body seen from the ventral aspect. Young dog. The course of the branches derived from the vertebral arteries on each side is shown in the upper part of the radiograph. These wind around the ventro-lateral aspects of the body to gain its ventral surface. Branches to the epiphysis are shown on the upper left-hand side. The stem of the artery to the centrum is seen in the centre of the specimen.

Fig. 4. (Key A 1.2.) Lumbar vertebra, viewed from cranial aspect. Dog, aged 6 years. Branches of the intraspinal arteries to the pedicles and laminae are shown. Note the anastomoses with extraspinal branches derived from the vessels which supply predominantly the sacrospinalis muscles. Arteries to the centrum are also seen in the basivertebral canal.

Fig. 5. High power view from fig. 8. The clear area in the top right-hand corner of the illustration is the intervertebral disc (arrow). Note the distribution of branches adjacent to the disc on one side and the growth plate on the other. Metaphyseal loops are seen adjacent to the giant cell layers of the growth plate.

Fig. 6. (Key B 2.2.) Cervical vertebral body seen from the side. The neural arch has been discarded. Mongrel dog, aged 6 months. Note the distribution of metaphyseal and epiphyseal arteries on the right side of the specimen. There is no anastomosis between these vessels across the growth plate.

Fig. 7. (Key A 1.0.) Lumbar vertebra viewed from cranial aspect. Dog, aged 5 years. The neural elements have been removed from the specimen. The course of the ventro-lateral extraspinal epiphyseal arteries is shown. The vessels in this specimen are not well filled, so that anastomoses are not shown.

Fig. 8. Low-power photomicrograph of lumbar intervertebral disc and adjacent vertebral bodies. Mongrel dog aged 6 months. The ventral surface of the disc is on the right of the picture. The specimen was injected with a mixture of equal parts of Micropaque and 10% Berlin Blue solution. The section was unstained (400μ, thick). In the upper epiphysis an extraspinal epiphyseal artery is shown and an intraspinal epiphyseal artery is seen entering the lower epiphysis.

Fig. 9. (Key C 3.1.) The dorsal surfaces of two lumbar vertebrae are shown. Puppy. The origins of the spinal arterial arcades are shown, particularly on the right side of the specimen. The close relationship between these arteries and the arcs formed by the longitudinal vertebral venous sinuses is shown, over the lower vertebra, where these veins appear partly filled. The delicate branches of the arteries seen crossing the intervertebral disc contribute to the rich anastomosis between the intraspinal arteries supplying the vertebral bodies

Fig. 10. (Key B 2.1.) Lumbar vertebral body viewed from side. Dog, aged 6 years. The tree-like pattern of the centrum artery is shown. Note the fine contribution of intra-spinal arteries to this system. The loosely knit anastomoses between epiphyseal and centrum arteries are clearly shown.

PLATE 3

Fig. 11. (Key B 2.2.) The sixth and seventh lumbar vertebrae and the first sacral segment are viewed from the side. Young dog. The contributions of intraspinal and extraspinal arteries to the supply of the centrum are shown. In the seventh lumbar vertebra the main vessel to the centrum is seen running in the basivertebral canal. Intraspinal epiphyseal arteries are seen in relation to the uppermost intervertebral disc in the illustration.

Fig. 12. (Key A 1.3.) Thoracic vertebral body viewed from cranial aspect. The neural elements have been removed. Adult dog. This illustrates the centrum artery pattern where the main parent vessel has an intraspinal origin.

Fig. 13. (Key C 3.2.) From the same specimen as illustrated in Pl. 2, fig. 3. This shows the tree-like pattern of distribution of the artery to the centrum. Note the epiphyseal arteries arising directly from the vertebral arteries in the lower part of the specimen.

Fig. 14. (Key B 2.0.) Lumbar vertebra viewed from the side. Adult dog. In the upper half of the illustration intraspinal arteries are shown entering the pedicle and coursing outwards in the lamina to the region of the zygapophyseal joints. The arteries to the epiphyses and the centrum, derived from extraspinal sources, are shown in the lower half.

Fig. 15. (Key A 1.4.) Lumbar vertebra viewed from cranial aspect. Adult dog. The straight course of arteries within the transverse processes, spinous process and laminae are shown. Extraspinal arteries to the epiphyseal zone are also shown.

Fig. 16. The central artery of a lumbar spinous process is shown. Note its relation to the partly filled vein lying on its right side in the upper part of the picture.

Fig. 17. (Key C 3.1.) Lumbar vertebra viewed from behind. Dog, aged 4 years. The arcs of the longitudinal vertebral venous sinuses are shown. Towards the centre these veins are filled incompletely. The extravertebral veins are seen in the upper right and left corners of the radiograph. On the right side, the connexions of the pedicle vein are shown.

Fig. 18. (Key A 1.0.) Lumbar vertebra viewed from cranial aspect. Dog, aged 12 years. The delicate arborizing, bilaterally symmetrical veins drain by single stems into the longitudinal vertebral venous sinuses on each side. The connexions of veins draining the lamina and zygapophyseal joint areas are shown where there is a rich anastomosis between extravertebral and spinal canal veins.

Fig. 19. (Key A 1.2.) Thoracic vertebra viewed from cranial aspect. Dog, aged 6 years. Bilateral basivertebral veins are shown converging in the centre of the vertebral body.

PLATE 4

Fig. 20. (Key A 1.2.) Lumbar vertebra viewed from cranial aspect. Dog, aged 4 years. This shows the union of bilateral basivertebral veins in the centre of the vertebra—with the emergence of a large left-sided, and a smaller right-sided branch on the ventral surface of the vertebral body.

Fig. 21. (Key A 1.2.) Cervical vertebra viewed from cranial aspect. Dog, aged 12 years. The single basivertebral vein is shown, bifurcating just before emerging from the ventral aspect of the vertebral body. The passage of veins from the transverse process and neural elements towards the pedicles is shown.

Fig. 22. (Key B 2.0.) Lumbar vertebra viewed from the lateral aspect. The base of the pedicle has been cut across and the vertebral body is shown alone. Dog, aged 12 years. The thick vein seen in the upper right segment of the specimen is running in the pedicle. It joins the longitudinal vertebral venous sinus which is running below and parallel to it, in the region of the intervertebral foramen. The longitudinal vertebral venous sinus appears as a broken heavy line in the illustration. The basivertebral vein connects it with the extravertebral veins, on the ventral surface of the vertebra. Veins from the centrum drain into the basivertebral vein, joining it at right angles. The vein draining the 'epiphyseal' zone of the vertebral body is seen in profile, adjacent to the disc on the right side of the specimen. Note the single stem passing directly into the longitudinal vertebral venous sinus.

Fig. 23. (Key B 2.1.) Another section from the specimen illustrated in fig. 22. The pattern of veins draining the centrum is clearly shown. They enter the basivertebral vein at right angles.

Fig. 24. (Key C 3.2.) Lumbar vertebra viewed from behind. Dog, aged 4 years. The rosette appearance made by the veins draining into the basivertebral vein is shown. Note the main stems of the veins draining the transverse processes. These join the pedicle vein already illustrated in figs. 17 and 22.

Fig. 25. (Key C 3.3.) Lumbar vertebra viewed from behind. Dog, aged 4 years. This shows the rosette pattern of veins draining the centrum. The larger branches enter the bilateral basivertebral veins at right angles.

Fig. 26. (Key B 2.4.) Another section from the same specimen as illustrated in figs. 22 and 23. The outline of the partly filled longitudinal vertebral venous sinus is shown in the upper right hand corner of the photograph, marked with an arrow. The 'epiphyseal' vein is shown entering it.

Fig. 27. (Key A 1.5.) Lumbar vertebra viewed from cranial aspect. Puppy. The grey halo within the vertebral body is made up by the fine veins draining the centrum; they form a background against which the epiphyseal veins are seen. The adult pattern can be made out with some difficulty. Note the many fine branches which are disposed radially around the vertebral body, draining directly into extravertebral veins.

Fig. 28. (Key C 3.0.) Cervical vertebra seen from behind. Adult dog. The vein draining the spinous process is shown bifurcating where the process joins the lamina. Note the confluence of these diverging branches with veins in the pedicles.

Plate 5

Fig. 29. Thoracic spinous process. Dog, aged 4 years. The venous drainage of the spinous process is shown. Note the number of anastomoses between fine branches of this central vein and veins in the periosteum and surrounding tissues.

Fig. 30. (Key B 2.2.) Lumbar vertebral body alone, seen from the side. Puppy. The basivertebral vein is shown in the centre of the specimen. Note the delicacy and concentration of the branches draining into it from the centrum. The course of the epiphyseal veins towards the spinal veins is shown; the latter are not shown in the radiograph.

ELECTRON MICROSCOPY OF THE DEVELOPING KIDNEY: AN INVESTIGATION INTO THE FINE STRUCTURE OF THE MESONEPHROS AND METANEPHROS OF THE RABBIT

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INTRODUCTION

The problem of the possible function of the mesonephros has attracted the attention of many workers. Leeson & Baxter (1957) reviewed the relevant literature and reported the results of a correlated histological and histochemical study of the mesonephros and metanephros in the rabbit which indicated a functional capacity of the mesonephros in this animal. Leeson then (1957) working on the same form with the electron microscope was able to demonstrate a distinct similarity in the fine structure between the fully developed 17-day mesonephric nephron and that of the metanephros.

This investigation has now been extended and the development of the mesonephros and metanephros in the rabbit studied using similar electron microscope techniques. Hall (1954), Pease (1955), Rhodin (1954) and Yamada (1955) have published accounts of the fine structure of the adult metanephros in animals and investigated its development. The electron microscopic appearance of the adult metanephric nephron is now well-established although a few problems remain, and are the source of disagreement, notably that of the presence or absence of a 'mesangium'. The major part of this report is concerned with the mesonephric glomerulus, and to a less extent with the mesonephric tubule whilst the metanephric glomerulus and tubule are discussed only in so far as they resemble, or differ from, their mesonephric counterparts.

MATERIALS AND METHODS

Rabbit embryos of 13, 15, 16 and 19 days conception age were studied as well as kidneys of neonatal and adult animals.

The fixation of tissues and its processing for electron microscopy followed more or less standard methods. Buffered 1 % osmium tetroxide (Palade, 1952), Dalton's bichromate fixative (Dalton, 1955) were routinely used. In some cases buffered potassium permanganate (Luft, 1956) was employed with satisfactory results. Tissue blocks were infiltrated with butyl-methyl methacrylate mixture with the stabilizer removed and using 1 % w/v benzoyl peroxide as a catalyst. After placing in gelatin capsules, the methacrylate was polymerized by treatment for 24 hours at a temperature of 60° C, which temperature has been shown (Borysko, 1956) to cause less polymerization change than 48° C. Ultra-thin sections were cut, either with an 'M.S.E.' (Cook & Perkins) or a 'Sims-Leeson' ultramicrotome (Sims & Leeson 1957). The sections were examined in an R.C.A. E.M.U. 2*a* microscope.

FINDINGS

(*a*). 13-*day Mesonephros*

Embryos from two litters were examined, the size of the embryos being from 8·1 to 9·2 mm. C.R., and 10·0 –11·0 mm. C.R., measured before fixation. At this stage, the glomeruli are large and easily recognized, but relatively simple in structure (Pl. 1, fig. 1). The parietal epithelium of the glomerular capsule is already attenuated and rests upon a thin but well-defined basement membrane. At the tubular neck, the low cuboidal type of cell of the capsule changes to a more columnar type of cell of the tubule. The lamina densa or basement membrane, as described in the adult metanephros (Hall, 1954, and others) is present at this stage as a thin layer applied more closely to the epithelial than the endothelial cells (Pl. 1, fig. 2). The visceral epithelial cells or podocytes are relatively undifferentiated and show overall a greater electron density than do the endothelial cells (Pl. 1, fig. 1). In the more rostral part of the mesonephros, however, the glomeruli are more fully differentiated and pedicels, or foot-processes, of the podocytes are formed and attached to the lamina densa (Pl. 1, fig. 2). The endothelial cells lining the glomerular capillaries are not attenuated to the same extent as in the adult metanephros (in which structure they are referred to as the 'lamina attenuata') and show large mitochondria with marked cristae mitochondriales but a very electron lucid matrix. Indeed, in many of the glomeruli, these endothelial mitochondria appear degenerate. Nucleated red cells are seen in the capillaries of some of the glomeruli.

The uriniferous tubule of this stage shows cells which are very simple in structure, and it is difficult to distinguish proximal from distal segment. The tubule is supported by a basement membrane. In some cases it is difficult to distinguish intercellular plasma membranes (Pl. 1, fig. 4), but in others the plasma membranes are prominent (Pl. 1, fig. 3).

(*b*) 15-*day Mesonephros*

The variation in size of these embryos was from 14·8 to 17·0 mm. C.R. length. Throughout the length of the mesonephros, all glomeruli are fully differentiated and show the three layers of the 'filtration barrier' as recognized in the adult metanephros (Pl. 1, figs. 5, 6).

The similarity between the mesonephric and metanephric glomerulus is very marked. The mesonephric podocyte shows marked endoplasmic reticulum as noted in the 13-day stage and the lamina densa is not so closely defined and not so thick in the mesonephros as in the metanephros.

The epithelial cells of the proximal segment of the uriniferous tubule are columnar in type, resting on an extracellular basement membrane and have a brush border composed of numerous microvilli. In some cases, but not all, there are deep intercellular clefts between the cells extending to the basement membrane. In the cytoplasm, the usual organelles are present, i.e. nucleus with its double nuclear membrane, mitochondria, Golgi apparatus and endoplasmic reticulum. In the apical part of the cell are present small vesicles and large vacuoles which may be identical with the 'fluorescent granules' (Sjöstrand, 1945), and in the base of the cells can be seen a few large, homogeneous granules. Infoldings of the basal plasma membrane (β-cytomembranes) are sparse. In the distal segment, again in some cases, there are deep intercellular clefts (Pl. 1, fig. 7). At the luminal border, small, thick microvilli are present (Pl. 2, fig. 9), but no vesicles are apparent. The nucleus is situated usually more centrally in the cell and endoplasmic reticulum is sparse. Infoldings of the basal layer of the plasma membrane are seen in some cells but never are so profuse as in the adult metanephros. The adjacent plasma membranes between two tubular cells may show complex interdigitations (Pl. 2, fig. 8).

(*c*) 16-*day Mesonephros*

The embryos varied in size from 17·8 to 19·8 mm. C.R. length. In the glomerulus there is practically no change in appearance from the 15-day stage. However, perhaps in this stage more than in all others the extent and complexity of the endoplasmic reticulum in the podocytes is most marked (Pl. 2, fig. 10). There is no evidence of degeneration. In the proximal part of the tubule, the cells appear both a little more differentiated than in the earlier stage (Pl. 2, fig. 11) and show signs of probable degeneration. β-Cytomembranes are more pronounced than in the earlier stage, but some of the mitochondria show few internal cristae mitochondriales and there are outlines of possibly degenerate mitochondria in the cytoplasm (Pl. 2, fig. 12). The appearances in the distal segment are indistinguishable from the 15-day stage.

(*d*) 19-*day Mesonephros*

The range of size was from 28·2 to 30 mm. C.R. length. The greater part of the mesonephros at this stage shows little evidence of involution and only the most rostral nephrons show any features which are possibly degenerate (Pl. 2, fig. 13; Pl. 3, fig. 14). Leeson & Baxter (1957) have shown that at 19 days the most rostral one-fifth of the mesonephric body in the rabbit is degenerate. The glomeruli and distal segments of the tubules show appearances which in the main are indistinguishable from previous stages. However, podocytes of the most cranial glomeruli show vacuolization of the cytoplasm and disruption of the organelles. In the proximal segment, the β-cytomembranes appear more pronounced than in earlier stages (Pl. 3, fig. 15).

(*e*) *Neonate*

The rabbit metanephros at birth with light microscopy shows nephrons in all stages of development, those situated nearest to the medulla being the oldest and fully differentiated whilst those at the peripheral cortex are immature and in an early stage of development. This is confirmed by electron microscopy, the juxtamedullary glomeruli showing all the appearances of the adult ones (Pl. 3, fig. 16), and, indeed, being indistinguishable from them. Younger glomeruli near the surface show features similar to those previously reported for the human foetus (Kurtz, 1958), notably the absence of pedicels, the podocytes lying adjacent to the lamina densa, and the thick endothelial layer, i.e. without attenuation of the cytoplasm. Even in immature glomeruli, it is usual to find red blood corpuscles. The tubules associated with these immature glomeruli are also incompletely differentiated, the cells generally being of low columnar type, with a brush border of relatively short microvilli and with few infoldings of the basal layer of the plasma membrane (β-cytomembranes) (Pl. 3, figs. 17, 18). The electron-microscopic appearance of the immature glomerulus and tubule has been reported previously (Kurtz, 1958 and Clark, 1957, respectively).

(*f*) *Adult*

In the rabbit, the electron-microscopic appearances of the renal glomerulus and tubule are similar to those reported previously in numerous other animals, including man. However, no cell was found in the glomerulus which could not be identified as epithelial, endothelial or as a blood cell, i.e. there was no evidence for the presence of a mesangium (Pl. 3, fig. 19).

DISCUSSION

With the electron-microscope, the structural details of the adult mammalian glomerulus (and the glomeruli of lower animals) have been shown to be complex, the structure obviously being associated with the function of ultra-filtration. It is established that the endothelial lining of the glomerular capillary is either very attenuated or even perforated although the actual perforations seen by some investigators may be a fixation artifact. The visceral epithelial cells or podocytes are supported and separated from the attenuated endothelial cytoplasm by an extracellular basement membrane which at the resolution obtained by present microscopes appears homogeneous. These epithelial cells cover the capillary loops in an octopus-like fashion, having large processes spreading out from the central, nucleus-containing cytoplasm, from which processes numerous smaller ones pass centrally to be attached to the outer surface of the basement membrane. Between these smaller foot-processes or pedicels are slits of the filtration space into which in life the glomerular filtrate passes. The complex structural arrangement is also found in the fully-developed mesonephric glomerulus in the rabbit and it is reasonable to conclude that the mesonephric glomerulus is *capable* of a similar function to that of the metanephric, if we admit of a positive relationship between structure and function.

Much evidence has accumulated in the past for a capability of function of the mesonephros and metanephros before birth and this evidence has been recently reviewed (Leeson & Baxter, 1957). The present investigation presents further evidence.

A few differences between the rabbit mesonephros and metanephros as seen with the electron-microscope should be mentioned. The extent and complexity of the endoplasmic reticulum in the mesonephric podocytes is to be noted. This complexity remains in a 19-day glomerulus until just before degeneration of the mesonephros occurs. The lamina densa in the mesonephric glomerulus is less clearly defined and less thick than in the adult (350–450 Å in the mesonephros, 400–600+ Å in the metanephros). It is tempting to associate this thinness of the basement membrane with the low filtration pressure which is known to be present in the mesonephric glomerulus. In the tubule in the earlier stages examined, the presence of deep intercellular clefts is peculiar, and is similar to that previously reported for other tissues (Walker, 1958). This could possibly be an artifact consequent on fixation or embedding, but it has been seen in several embryos, and it is notable that adjacent tubules in the same section will differ, one showing the intercellular clefts and one showing the more usual appearance. In the apical cytoplasm of the tubular cells of the proximal segment the presence of large vesicles has been reported previously in the 17-day rabbit embryonic mesonephros (Leeson, 1957) and has been noticed again in younger embryos in this investigation. These vesicles may be identical with the fluorescent granules previously described (Sjöstrant, 1945) and later identified in an electron microscope study (Sjöstrand & Rhodin, 1953).

The infoldings of the basal plasma membrane (β-cytomembranes) have been reported as occurring not only in renal tubules but also in other structures, e.g. chorioid plexus, epithelia of submaxillary glands and ciliary body. Pease (1956) concluded that this was a mechanism by which basal cell surfaces might be expanded, presumably to aid transference of fluid. In the fully-formed rabbit mesonephric tubule, these basal infoldings are prominent although perhaps not so extensive as in the adult metanephric tubule.

SUMMARY AND CONCLUSIONS

This investigation has established a remarkable similarity in structure between the mesonephros and metanephros in the rabbit as seen with the electron microscope, particularly with regard to the glomerulus. It is concluded that the complexity of structure of the rabbit mesonephros indicates a capability of function of this organ in the embryo.

This investigation was performed whilst I held a British Medical Association Research Scholarship and it is a pleasure to record my gratitude to the Association. I am particularly indebted to Prof. J. S. Baxter of the Department of Anatomy, University College, Cardiff, who initiated my interest in this problem and who originally provided me with facilities to learn the techniques of electron microscopy. Dr F. Jacoby and he have critically reviewed this manuscript. My thanks are also due to Mr A. L. Sims and Prof. Peterson of the Physiology Department at University College, Cardiff, for the manufacture and loan of a Sims-Leeson ultramicrotome: to Prof. Duckworth of this Department for advice and facilities, and to Mr G. T. Woodward of the Department of Physics, University of Toronto, for valuable technical assistance with the electron microscopy.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. 13-day mesonephric glomerulus. The capsular space (*S*) between the parietal (*P*) and visceral (*V*) epithelial cells is shown with the lumen (*L*) of a glomerular capillary, lined by endothelial cells (*B*). Mitochondria (*M*), endoplasmic reticulum (*E*), and nuclei (*N*) are clearly seen. ×7200.

Fig. 2. 13-day mesonephric glomerulus. This micrograph is of a more fully developed nephron showing an endothelial cell nucleus (*N*), and the lamina densa or basement membrane (*D*) to which are attached pedicles (*F*) of a podocyte or visceral epithelial cell (*V*). ×18,000.

Fig. 3. 13-day mesonephric tubule, basal region. The basement membrane of the tubule is shown (*A*) outside the plasma membranes (*C*) of the tubule cells. Plasma membranes between adjacent tubules are well marked (arrowed). The nuclear membrane (*NM*) is seen to be double and mitochondria (*M*) with internal membranes are clearly seen. ×45,200.

Fig. 4. 13-day mesonephric tubule. The base of the tubule is below and nuclei (*N*) and mitochondria (*M*) are seen. The presence of plasma membranes between the cells is doubtful. ×3600.

Fig. 5. 15-day mesonephric glomerulus. A survey showing parietal and visceral (*V*) epithelial cells, capsular space (*S*), endothelial cells (*B*) and the lumina of two capillary loops (*L*). ×2700.

Fig. 6. 15-day mesonephric glomerulus. An endothelial cell nucleus (*N*), surrounded by the cytoplasm, lines the lamina densa (*D*) on to the external surface of which are attached numerous pedicels (*F*) of a visceral epithelial cell (*V*), the cytoplasm of which contains strands of endoplasmic reticulum. ×18,000.

Fig. 7. 15-day mesonephros, distal segment of the tubule. Intercellular clefts (*W*) extending to the basement membrane (*A*) are very obvious. The lumen (*L*) of the tubule is shown. ×7200.

PLATE 2

Fig. 8. 15-day mesonephros, distal segment. The basement membrane of the tubule (*A*) is shown, the nuclei (*N*) of two tubular cells, numerous mitochondria (*M*) with their internal membrane systems, and the complex convolutions of the plasma or cell membranes (*C*) between the two cells. ×25,100.

Fig. 9. 15-day mesonephros, distal segment. The lumen (*L*) of the tubule is above, projecting into which are numerous small processes, microvilli, of the tubule cells. Intercellular clefts (*W*) are present but not so well seen as in fig. 7. ×2700.

Fig. 10. 16-day mesonephric glomerulus. The cytoplasm of the visceral epithelial cell (*V*) contains numerous mitochondria (*M*), strands of endoplasmic reticulum (*E*) and the cell has many pedicels (*F*) attached to the lamina densa (*D*), which in turn is lined by a thin sheet of endothelial cytoplasm (*B*). Part of a red blood cell (*R*) in the capillary lumen (*L*) is shown. ×12,000.

Fig. 11. 16-day mesonephros, proximal segment. The apex of the tubular cell shows many microvilli (*T*), which form the brush border, numerous small vesicles and a few large vacuoles, part of one being shown (*U*). ×26,100.

Fig. 12. 16-day mesonephros, proximal segment. The central part of the cytoplasm of a tubular cell is shown with nucleus (*N*), mitochondria (*M*), some mitochondrial 'outlines' (*O*), and the Golgi complex (*G*). ×18,000.

Fig. 13. 19-day mesonephric glomerulus. The capillary lumen is below, lined by a thin sheet of endothelial cytoplasm (*B*). Attached to the lamina densa (*D*) are numerous pedicels (*F*) of a visceral epithelial cell (*V*) in the cytoplasm of which is a large mitochondrion (*M*). ×42,900.

PLATE 3

Fig. 14. 19-day mesonephric glomerulus. This rostral glomerulus is degenerate, the visceral epithelial cells (*V*) showing numerous large vacuoles. Pedicels (*F*), lamina densa (*D*) and endothelial cell (*B*) appear intact. The capillary lumen is shown (*L*). ×26,100.

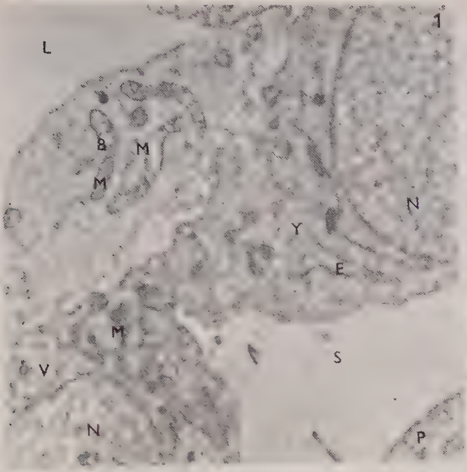
Fig. 15. 19-day mesonephros, proximal segment. The basement membrane (*A*) is not clear but numerous mitochondria (*M*) with their internal membrane systems are well seen, some surrounded by infoldings of the basal layer of the plasma membrane (*C*). ×26,100.

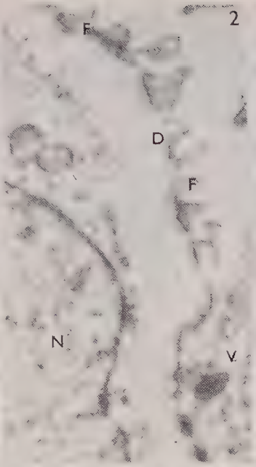
Fig. 16. Neonate glomerulus. The endothelial cell nucleus (*N*) is surrounded by little cytoplasm which becomes attenuated to line the capillary lumen (*L*). The lamina densa (*D*) and numerous pedicels (*F*) are shown. ×12,000.

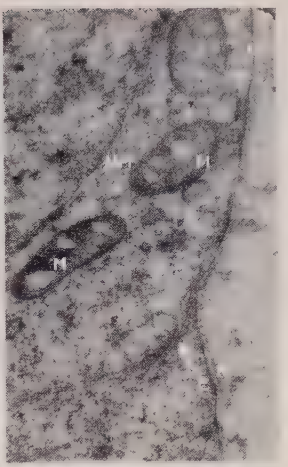
Fig. 17. Neonate, proximal segment. Parts of two nuclei (*N*) are seen between which are numerous mitochondria (*M*) surrounded by infoldings of the basal layer of the plasma membrane (*C*). ×36,000.

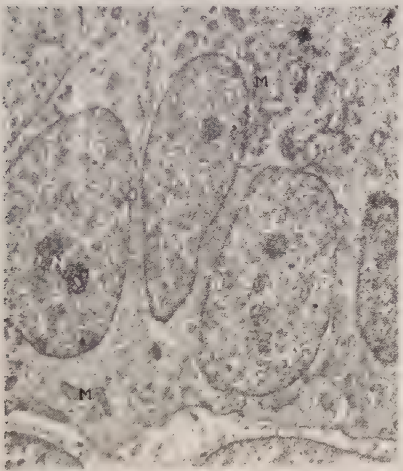
Fig. 18. Neonate, distal segment. The plasma membrane (*C*) is infolded from the basement membrane (*A*) to surround mitochondria (*M*). Part of a nucleus (*N*) is shown with its double nuclear membrane. ×26,100.

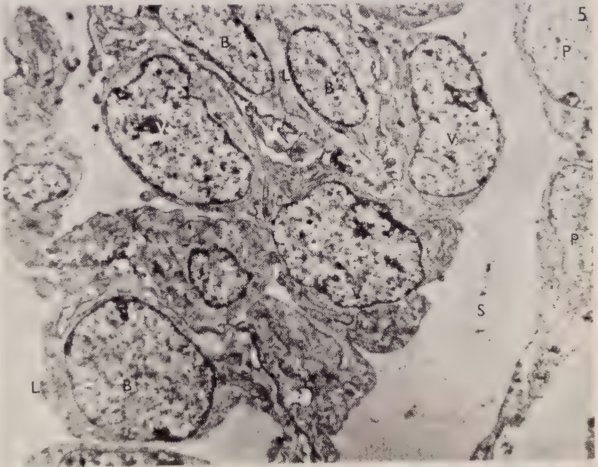
Fig. 19. Adult glomerulus. This micrograph shows the complexity of structure. Capillary lumen (*L*) is lined by endothelial cytoplasm (*B*). The lamina densa (*D*) is thicker than in the mesonephros and to it are attached pedicels (*F*). ×18,000.

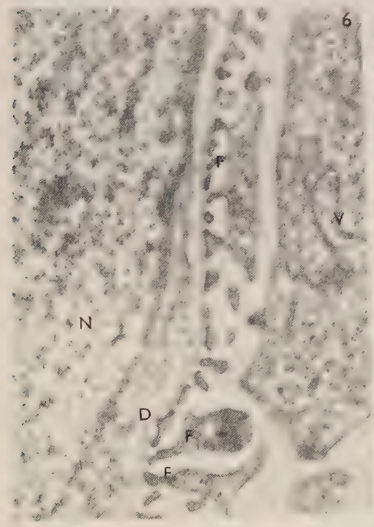


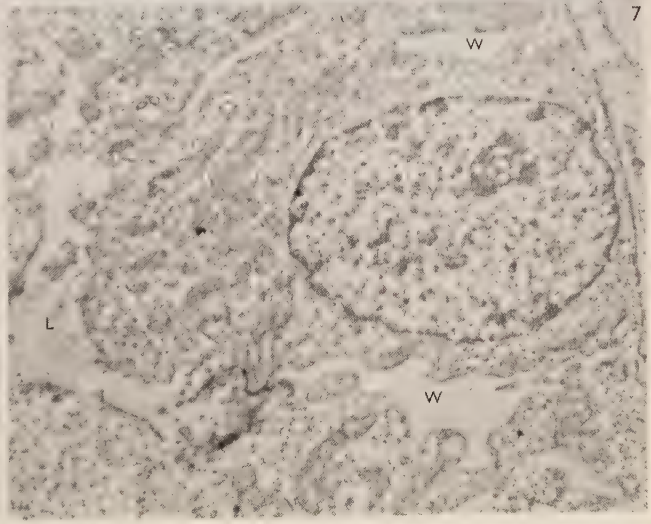












LEESON—ELECTRON MICROSCOPY OF THE DEVELOPING KIDNEY

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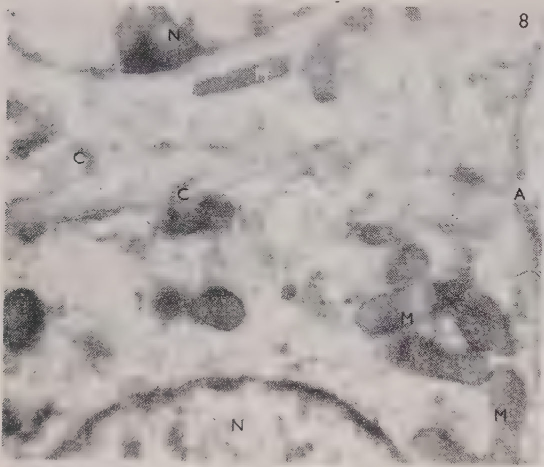
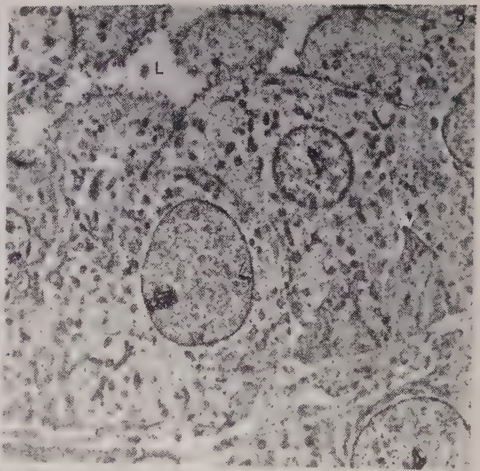
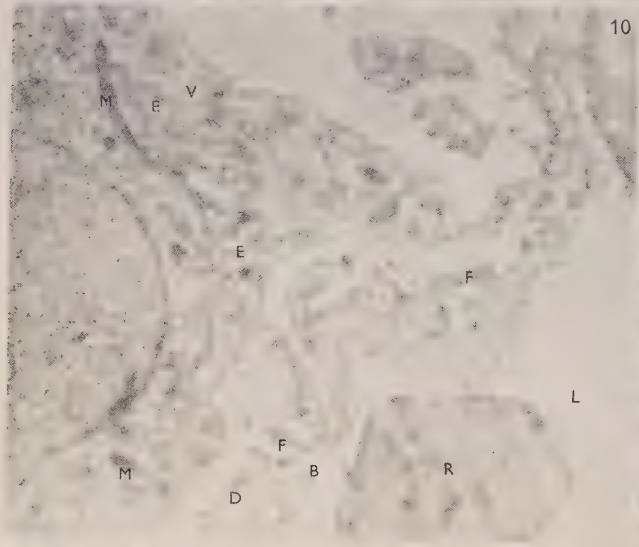
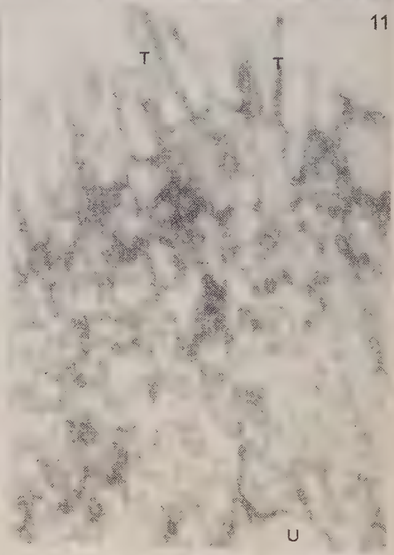
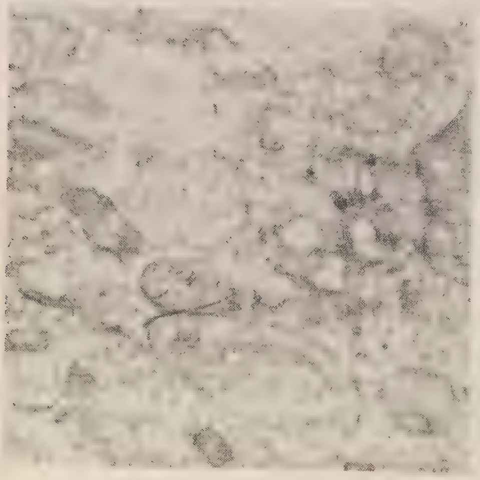
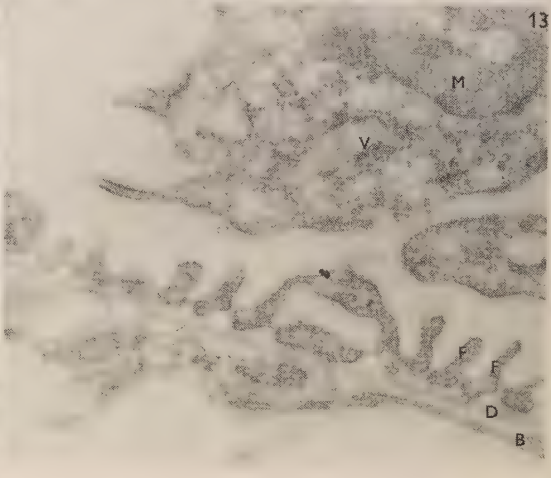




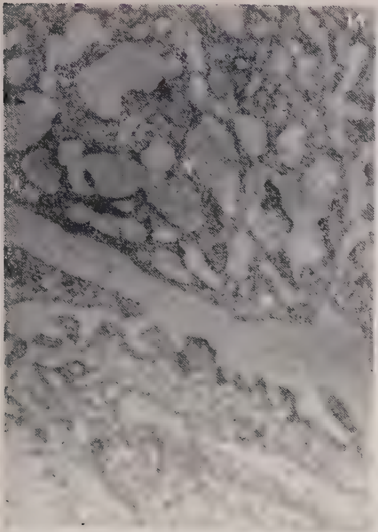


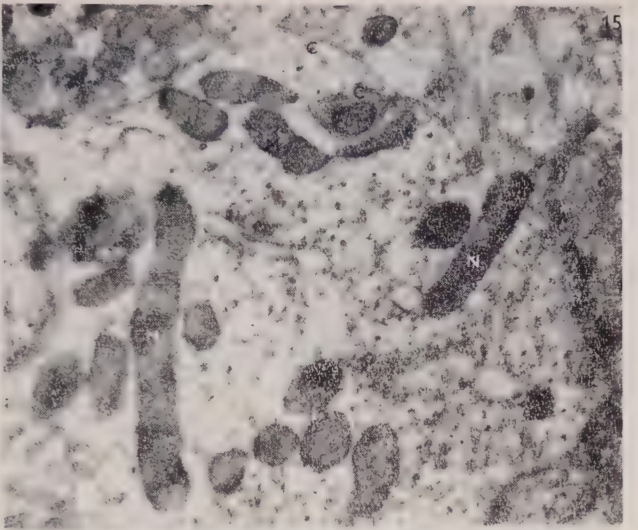


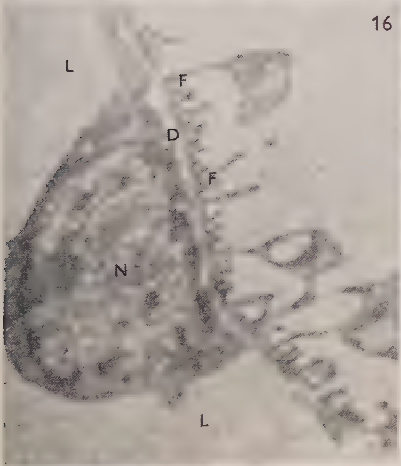


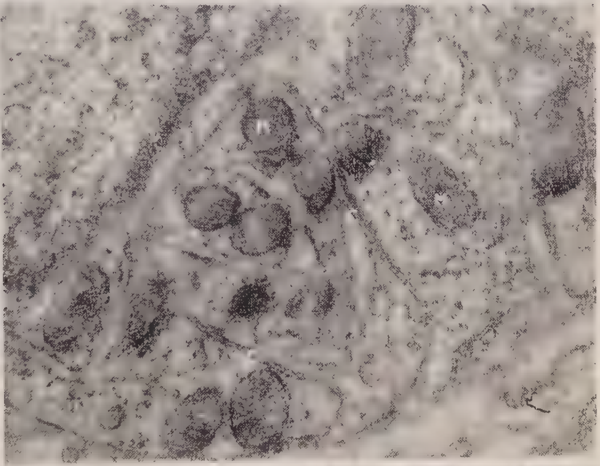


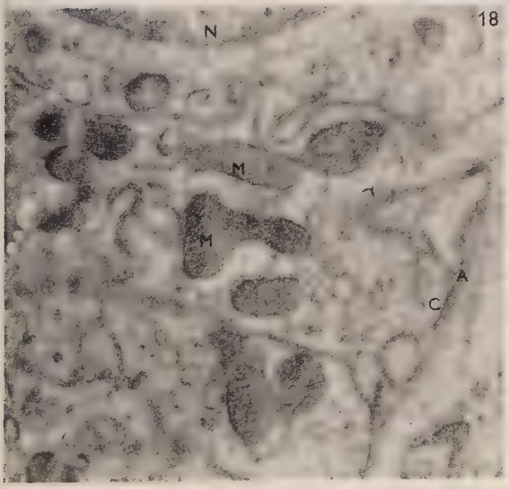
LEESON—ELECTRON MICROSCOPY OF THE DEVELOPING KIDNEY

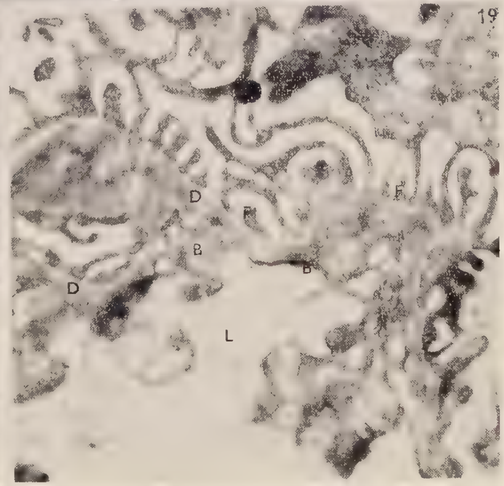












LEESON—Electron microscopy of the developing kidney

THE GUBERNACULUM TESTIS OF THE PIG (*SUS SCROPHA*)

By K. M. BACKHOUSE and H. BUTLER

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John Hunter (1762) first described the structure, which he named the gubernaculum testis, 'a substance which runs down from the lower end of the testis to the scrotum.' He considered it to be 'vascular and fibrous, and covered by the fibres of the cremaster or musculus testis which is placed immediately behind the peritoneum; this is not easily ascertained in the human subject, but it is very evident in other mammals, more especially in those whose testicles remain in the cavity of the abdomen after the animal is full grown.'

Since Hunter, the gubernaculum has been described repeatedly. In general the earlier authors, Paletta (1777), Wrisberg (1779), Vicq d'Azyr (1780), Martin (1780) and Brugnoni (1785) described a peritoneal covered structure, extending from the caudal pole of the testis to the scrotal floor, containing fibrous and/or muscular tissues, yet being soft and jelly-like. In the course of time the jelly-like nature of the gubernaculum testis became ignored and it is currently described as a fibro-muscular cord or band (Hamilton, Boyd & Mossman, 1952; Patten, 1948). Confusion was caused by Seiler's (1817) studies on a variety of adult mammals, each having its own peculiarities of testicular anatomy and physiology, because he uncritically applied facts derived from these studies to the gubernaculum testis of the human foetus. Inaccurate observations on adult rodents (particularly the *Sciuridae*) led him to believe that the function of the cremaster muscle is to pull the testis from the abdominal cavity into the scrotum: hence he assumed that the 'inverted cremaster' of the human foetus behaved in a like manner.

Seiler's views received support from Curling (1841) who described a layer of well developed striped muscle fibres surrounding the main bulk of the soft transparent and gelatinous gubernaculum. He believed that this muscle layer, with its various distal attachments, is gradually everted during testicular descent and persists as the cremaster muscle.

However, by that date the views of Cloquet (1817) and Carus (1827) had gained currency, viz. that the cremaster muscle is formed mechanically by the passage of the testis through the abodominal wall; indeed this idea that the testis or its associated gubernaculum burrowed its way through the layers of the abdominal wall dragging them with it to form the coverings of the cord and testis is still to be found in current textbooks (Keith, 1948).

The statement made by Curling that 'the testicle, therefore, does not pass directly and abruptly into a pouch prepared to receive it, but carries the peritonaeum with it. . . ' is at variance with the findings made in the present study in the pig in which the processus vaginalis does in fact invade the gubernacular tissue in advance of the testis.

Lockwood (1887–88) attempted to equate the positions of the distal terminations of the human gubernaculum testis with those sites commonly occupied by an ectopic testis. He admitted that he could not find all the 'tails' of the gubernaculum testis which he postulated in the foetus, but insisted that they must exist because of the various sites of occurrence of ectopia testis and the presence of a fibrous band tethering such a testis to its abnormal site. Lockwood ignored the jelly-like consistency of the gubernaculum testis and referred to it as a fibro-muscular cord or band.

In view of the discrepancies in the accounts of the gubernaculum it was decided to make a thorough investigation of its development and structure in pig embryos and foetuses. This animal was chosen for these reasons: (1) it was possible to obtain abundant material during the critical period of testicular descent; (2) the gubernaculum testis is large; (3) there is precocious development of the processus vaginalis, and the cremaster muscle consists only of a single lateral fasciculus, thus greatly facilitating morphological analysis.

MATERIALS AND METHODS

Over 100 specimens of pig embryos and foetuses ranging in size from 6 mm. to 350 mm. crown-rump (C.R.) length were examined macroscopically and histologically. Many dissections of unfixed specimens of the larger foetuses were made within 2–3 hours of the mother's death. Comparable stages in the sheep (*Ovis aries*) were also examined.

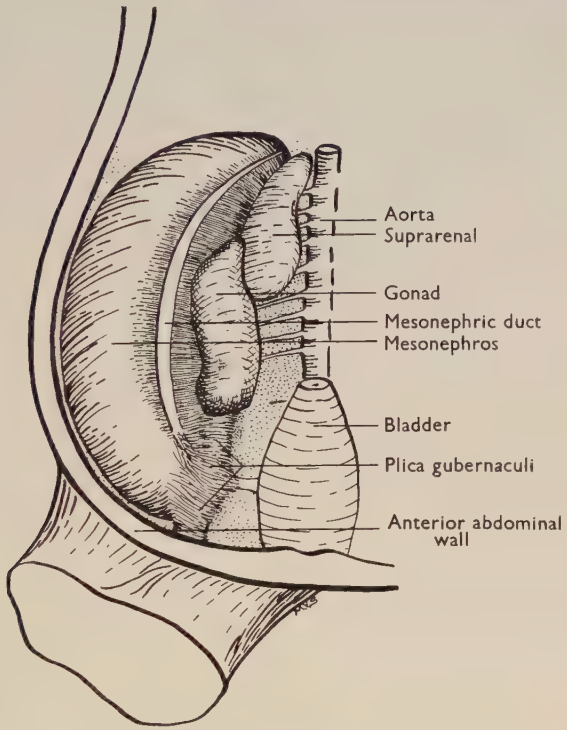
OBSERVATIONS

1. *Embryos of* 20–30 *mm.* C.R. *length*

A prominent feature of early pig embryos is the very large mesonephros whose caudal pole is in contact with the inguinal region of the anterior abdominal wall. The gonad develops at the cranial end of the mesonephros and the size of the latter organ appears to delay the formation of that part of the gubernaculum testis which extends from the gonad to the anterior abdominal wall. A strip of cuboidal coelomic epithelium overlying a thin layer of undifferentiated mesenchyme, the precursor of the gubernaculum testis within the abdomen, extends from the gonad, across the mesonephros and umbilical artery, to the inguinal region. This mesenchyme is continuous with a core of mesenchyme which demarcates the future inguinal canal within the differentiating abdominal wall muscles and which, in turn, is continuous externally with the mesenchyme in the genital swellings. Thus the precursor of the gubernaculum testis is a continuous strip of mesenchyme extending from the caudal pole of the gonad to the interior of the genital swelling. As yet, its abdominal part does not form a prominent intra-abdominal ridge.

2. *Embryos of* 30–50 *mm.* C.R. *length*

The mesonephros has grown in size but not so much as the body wall, and the caudal pole of the mesonephros moves away from the inguinal region of the anterior abdominal wall. The testis, still situated well cranialwards on the mesonephros, now possesses a well-defined mesorchium. The mesenchymatous continuum, linking the gonad with the scrotal pouch through the anterior abdominal wall musculature, exhibits marked growth, particularly in the abdomen. Within the abdomen the gubernaculum testis now forms a well-defined ridge, the *plica gubernaculi* (the so-called ligamentum inguinale of Klaatsch, 1890), projecting into the abdominal cavity and carried on a mesentery. Cranially the plica gubernaculi merges with the mesorchium at the caudal pole of the testis and is also continuous with the mesenchyme surrounding the mesonephric duct (Text-fig. 1). Thus a part of the mesonephric duct is contained within the gubernaculum. The plica gubernaculi then crosses the caudal pole of the mesonephros and the umbilical artery to reach the inguinal region. That part of the plica gubernaculi overlying the mesonephros is less prominent than that between the caudal pole of the mesonephros and the anterior abdominal wall. The processus vaginalis commences to form around the junction of the plica gubernaculi and the gubernacular mesenchyme in the site of the future inguinal canal, as an evagination of the coelomic cavity into the gubernacular mesenchyme of the abdominal wall. This evagination only extends around three sides of the column, leaving a mesentery in continuity with that of the already formed plica gubernaculi which therefore appears to enter the anterior abdominal wall beneath a prominent peritoneal covered lip. At the point of invasion, the coelomic cells are cuboidal and mitotic figures may be seen.



Text-fig. 1. Posterior abdominal region of a pig embryo of 40 mm. C.R. length showing the gubernaculum testis within the abdomen.

In a 38 mm. C.R. length embryo (Pl. 1, fig. 1), the plica gubernaculi between the lower pole of the mesonephros and the blind tip of the processus vaginalis is approximately 0·2 mm. long of which 0·1 mm. lies within the processus vaginalis: in an embryo of 45 mm. c.r. length the equivalent distances are now 0·6 mm. and 0·15 mm. respectively (Pl. 1, fig. 2).

That part of the gubernaculum testis within the inguinal canal is a mass of relatively dense mesenchyme into which the processus vaginalis grows. It is separated from the well-differentiated abdominal wall muscles by a small amount of looser mesenchyme. As yet, the processus vaginalis has only penetrated for about one-fifth of the total length of the inguinal canal. Beyond the processus vaginalis, the solid mass of mesenchyme is continuous, through the external inguinal ring, with subcutaneous mesenchyme between the abdominal and thigh muscles. Here the gubernaculum testis is less well defined since the subcutaneous tissues have not yet differentiated but it can be identified as a strand of relatively dense mesenchyme surrounded by looser tissue. This strand is, in turn, continuous with a globular mass of relatively dense mesenchyme which forms the central core of the developing scrotum (Pl. 1, fig. 3).

From about 40 mm. c.r. length onwards, the cremaster muscle fibres are apparent and differentiate from the gubernacular mesenchyme contained within the inguinal canal. Initially, this short fasciculus of fibres is entirely contained within the inguinal canal and neither its proximal nor distal attachments are apparent (Pl. 1, fig. 2).

3. *Embryos of* 50–100 *mm.* c.r. *length*

During this period involution occurs in the caudal pole of the mesonephros which underlies the cranial part of the plica gubernaculi and this is associated with an increase in size of the cranial part of the gubernacular ridge. In an 83 mm. c.r. length foetus, the portion of the plica gubernaculi between the testis and the caudal pole of the mesonephros is 2 mm. long and the portion caudal to the mesonephros, where the distal half lies within the processus vaginalis, is 2 mm. long (Pl. 1, fig. 4). Thus the length of the plica gubernaculi from the testis to the internal ring is 3 mm., and a further 1 mm. to the blind tip of the processus vaginalis.

The processus vaginalis continues to extend further into the inguinal canal but does not yet emerge from the external inguinal ring. The lip at the entrance to the processus vaginalis now forms the long valve-like flap found in the adult animal. The mesenchyme of that part of the plica gubernaculi situated within the processus vaginalis has a more densely staining ground substance than the rest of the gubernaculum. The cremaster muscle has increased in size and its origin from the inguinal ligament, lateral to the external ring, is now apparent. The muscle is confined to the inguinal canal, and no fibres run cephalically towards the testis.

Beyond the blind tip of the processus vaginalis the inguinal canal contains a solid mass of mesenchyme which is continuous, through a strand of mesenchyme in the groin, with the intra-scrotal part of the gubernaculum testis. The gubernacular mesenchyme of the groin and scrotum becomes more clearly delineated as a result of the differentiation of the surrounding tissues. The subcutaneous mesenchyme of the scrotum and lower abdominal wall contains numerous irregular spaces lined by flattened cells and containing an acidophilic coagulum, the superficial lymphatic plexus. Interlacing strands of fibrous tissue are also being laid down in the subcutaneous mesenchyme. The central core of gubernacular mesenchyme, however, remains unchanged.

4. *Foetuses of* 100–160 *mm.* C.R. *length*

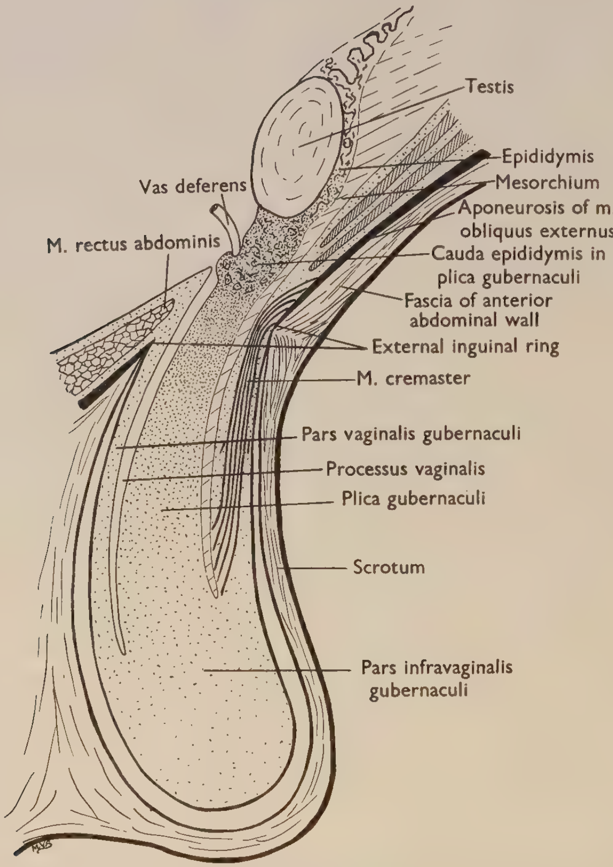
During this period the gubernaculum testis attains its completed form prior to testicular descent which normally occurs in foetuses of 190–200 mm. C.R. length. The mesonephros has now completed its involution leaving only its characteristic small remnants. As a result of this, the plica gubernaculi remains as a simple mesenchymatous column, carried on a mesentery continuous with the mesorchium and extending from the caudal pole of the testis to the distal end of the processus vaginalis. The testis and epididymis are carried on the apex of this column and that portion of the mesonephric duct embedded in it becomes convoluted to form the cauda epididymis. The portion of the gubernaculum testis distal to the external inguinal ring becomes much thicker, thus allowing the processus vaginalis to continue growing caudalwards towards the scrotum, within its substance. The processus vaginalis extends to emerge from the external inguinal ring at 110–120 mm. C.R. foetal length and it now becomes apparent that the cremaster muscle extends only so far distalwards as the processus vaginalis. The cremaster muscle differentiates along the line of attachment of the mesentery of the plica gubernaculi within the processus vaginalis and those two structures grow distally at the same rate.

With the exception of the peritoneum and of the striated muscle fibres of the cremaster, the simple mesenchymatous nature of the gubernaculum testis is retained even though changes occur in the surrounding abdominal wall and scrotal mesenchyme. In the inguinal canal, the gubernacular mesenchyme is continuous with the mesenchyme and differentiating connective tissue surrounding the abdominal wall muscles, and in this tissue the cremaster muscle runs distalwards from its inguinal origin into the gubernaculum testis. Distal to the external inguinal ring the superficial fasciae of the abdominal and scrotal walls become differentiated and form a connective tissue pocket containing undifferentiated gubernacular mesenchyme. Concurrently with this superficial tissue differentiation, a split occurs around the enclosed gubernacular mesenchyme, and extends, distally, around the floor of the scrotum and, proximally, to the external inguinal ring. The gubernaculum testis thus comes to lie free in the scrotum and if, from 140–150 mm. C.R. foetal length onwards the scrotum be opened, an elongated pyriform mass can be lifted out. This mass, however, remains attached to the tissues surrounding the inguinal canal. (Pl. 1, fig. 5; Text-fig. 2).

In the fresh state the gubernacular tissue is pink and has a soft semi-fluid consistency: after formalin fixation it is semi-opaque and gelatinous. It closely resembles Wharton's jelly of the umbilical cord.

The definitive gubernaculum testis

It is now convenient to consider the gubernaculum testis as a whole and to name its constituent parts (Text-figs. 2, 3). The gubernaculum testis is an elongated pyriform mass of mesenchyme extending from the caudal pole of the testis to the scrotal floor. Its abdominal part is a mesenchymatous column suspended on a mesentery, continuous cranially with the mesorchium, and containing the cauda epididymis. Distally, it continues as a mesenchymatous mass which extends through the inguinal canal and fills the scrotal sac; the processus vaginalis and cremaster muscle grow into this mass. As the processus vaginalis grows into the inguinal and scrotal parts of the gubernaculum, so does the abdominal gubernacular column extend within this mass to the distal extremity of the processus vaginalis. The term *gubernaculum testis* (Hunteri) refers to the whole mass of mesenchyme extending from the testis to the floor of the scrotum and in which the processus vaginalis and cremaster muscle develop. The gubernaculum testis can, however, be subdivided into:


Testis
Epididymis
Vas deferens
Mesorchium
Aponeurosis of m. obliquus externus
M. rectus abdominis
Cauda epididymis in plica gubernaculi
Fascia of anterior abdominal wall
External inguinal ring
M. cremaster
Pars vaginalis gubernaculi
Processus vaginalis
Plica gubernaculi
Scrotum
Pars infravaginalis gubernaculi

Text-fig. 2. Schematic representation of the gubernaculum testis shortly before testicular descent, showing its component parts and also its relationship with the surrounding structures.

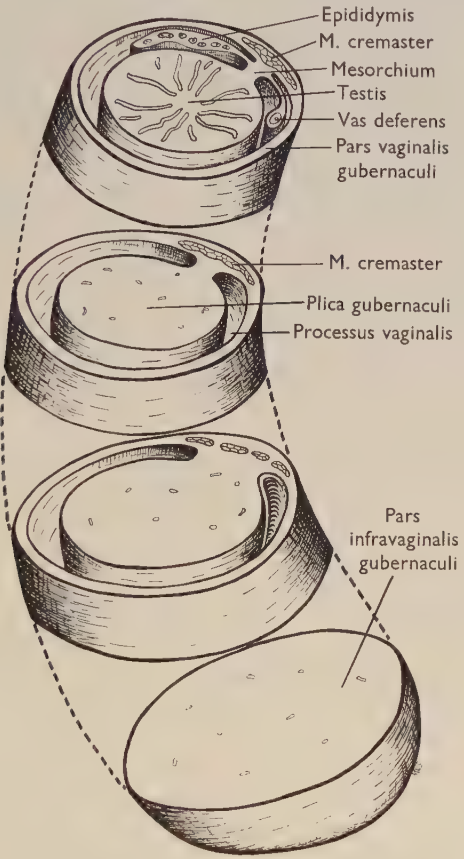
(i) *The plica gubernaculi* (*Seiler*), the intra-coelomic column of mesenchyme extending from the caudal pole of the testis to the distal extremity of the processus vaginalis.

(ii) *The pars vaginalis gubernaculi*, the thin layer of mesenchyme separated off by, and surrounding, the processus vaginalis. This layer is continuous with the plica gubernaculi through the root of the latter's mesentery. Distally it merges with

(iii) *The pars infravaginalis gubernaculi*, the solid mass of gubernacular mesenchyme distal to the processus vaginalis and into which the processus vaginalis and cremaster muscle grow as development continues.

Gubernacular changes attendant upon testicular descent

In the period immediately prior to testicular descent there are changes in the gubernaculum but its basic structure is not materially affected. The processus vaginalis and cremaster muscle continue their downward growth and by the time of testicular descent through the inguinal canal they have extended some two-thirds of the distance from the internal inguinal ring to the scrotal extremity of the gubernaculum. Other changes primarily affect the ground substance in various parts of the gubernaculum. The distal portion of the gubernaculum testis has contained, from quite early in its development, a considerable amount of semi-fluid ground substance and this has formed the bulk of the swelling in the scrotal region. As the time of testicular descent approaches the amount of ground substance increases making the distal end of the gubernaculum larger and more globular, so dilating the scrotum. Externally, the scrotum has a bluish translucency compared with the surrounding tissues. As the ground substance increases the gubernaculum testis ceases to grow in length but, being fixed by the pars vaginalis in the inguinal



Text-fig. 3. Schematic sectional representation of the gubernaculum testis, removed from the scrotum shortly after testicular descent.

canal and free in the scrotum, increase in size of the surrounding tissues leads to a withdrawal of the gubernacular tip from the scrotal floor. This withdrawal may amount to as much as 5·0 mm. in the pig.

Initially, the increase in ground substance is limited to the pars infravaginalis gubernaculi so that only the scrotum is dilated. As the foetus approaches 190 mm. C.R. length the increases in ground substance and cessation of growth in length of the gubernaculum testis gradually extends proximalwards into the plica gubernaculi. Prior to this phase in development, the plica gubernaculi has remained relatively thin, especially in the inguinal canal and immediately subjacent to the testis. Here the cellular density has been high and the amount of ground substance small compared with the more distal parts of the gubernaculum. As the increase in ground substance extends proximalwards, the pars infravaginalis et plica gubernaculi become swollen. This increase gradually extends proximally, first dilating the scrotum and then the channel of testicular descent, the inguinal canal being affected last of all. Thus the testis comes to form the apex of an elongated cone of swollen mesenchyme which holds the pathway along which the testis will descend.

The pars vaginalis gubernaculi does not undergo this general cessation of growth and increase in ground substance but continues to grow along with the surrounding body wall, and so carries the plica gubernaculi and the gubernacular tip distalwards. Thus the combination of distal growth of the pars vaginalis gubernaculi and the conical form of the plica gubernaculi carries the testis, situated on the apex of the plica gubernaculi, through the inguinal canal into the groin. While these changes are taking place in the gubernaculum, there is concurrently rapid growth of the vas deferens and testicular vessels to allow the testis to move.

Following the passage of the testis through the inguinal canal further changes take place in the gubernaculum. The processus vaginalis and the cremaster muscle continue to grow towards the scrotal floor and, as a result, the pars infravaginalis gubernaculi virtually disappears, *thus leaving only the plica et pars vaginalis gubernaculi.*

The plica gubernaculi does not actively shorten; its mesenchyme becomes involved by further growth of the cauda epididymis which eventually reaches the distal extremity of the gubernaculum. (Pl. 1, figs. 5, 6) The cauda epididymis thus approaches the scrotal floor.

Further testicular descent is brought about by lengthening of the scrotum, the pars vaginalis gubernaculi, the vas deferens and the testicular vessels. The testis does not approach the scrotal floor but both it and the scrotal floor move further away from the external inguinal ring. The mesenchyme of the plica gubernaculi becomes converted into the connective tissue of the cauda epididymis. The pars vaginalis gubernaculi which surrounds the processus vaginalis and contains the cremaster muscle becomes converted into the fascial covering of the testis and spermatic cord (Pl. 1, fig. 7) (the internal and external spermatic fasciae of man).

DISCUSSION

The major concept arising from this study is that of the mesenchymatous nature of the gubernaculum testis, a histological feature which persists in the plica gubernaculi so long as the structure remains as a recognizable entity. The gubernaculum testis can thus be defined as a band of mesenchyme extending *in the developing animal* from the caudal pole of the testis to the region of the future scrotum: it delineates the pathway along which the testis will migrate. As it is partly intra- and partly extra-abdominal, the intra-abdominal and extra-abdominal portions are often considered separately from a developmental point of view: certain authors have even described three developmentally fused portions to the structure, intra-abdominal, intramural and scrotal, with further subdivisions of the intra-abdominal portion (Felix, 1912; Moscowitz, 1936). Such complicated descriptions of the gubernaculum testis are considered however to be unnecessary and only in part true. A continuous band of mesenchyme exists in the track of the gubernaculum in the very early embryo, but only becomes a recognizable entity with the differentiation of the surrounding body wall structures, which thus delineate, in particular, the extra-abdominal portion of the gubernaculum lying in the inguinal canal and scrotal areas. Differences in cellular density can be seen within the inguinal canal and scrotum at different developmental stages, but these differences are related to the activity of the tissues and not to separate developmental entities. It is thus inaccurate to divide the extra abdominal portion of the gubernaculum into developmental intramural and scrotal entities, though these terms may be useful if limited to regional description.

The intra-abdominal portion of the gubernaculum develops in a manner different from the extra-abdominal portion but it need not be considered as a separate entity. In early stages (20 mm. C.R. length) the plica gubernaculi is slight, being only a thin band of undifferentiated mesenchyme underlying a primitive coelomic epithelium, but later this band forms a true ridge, the plica gubernaculi (ligamentum inguinale of Klaatsch). A continuous mesenchymatous band nevertheless runs from the testis to the scrotum even before the formation of the true ridge. The plica gubernaculi does not arise in the pig by the fusion of a plica inguinalis with a crista inguinalis as was postulated by Felix to be the case in man, but by a simple thickening along the length of the mesenchymatous band. In the pig, however, the close proximity of the mesonephros to the anterior abdominal wall which exists at the time of these changes would make the process scheme postulated by Felix unnecessary, whereas in man the gubernaculum testis must leave the mesonephros and pursue a course around the pelvic floor to reach the anterior abdominal wall.

The essentially continuous nature of the gubernaculum testis is emphasized by the gradual distal extension of the plica gubernaculi beyond the inner aspect of the anterior abdominal wall; by the growth of the processus vaginalis the plica gubernaculi eventually extends so far distalwards as the floor of the scrotum, but with no intrinsic change in the tissue within the plica and the pars infravaginalis gubernaculi. The described mode of extension of the processus vaginalis, by active coelomic epithelial invasion of the gubernacular mesenchyme, is at variance with the mode of extension of the lesser sac of peritoneum into the mesenchyme around the foregut as described by Kanagasuntheram (1957). He denied an active epithelial invasion and maintained that clefts formed in the mesenchyme, the clefts coalescing with each other and with the coloemic cavity. Such cleft formation has not been seen distal to the extending processus vaginalis.

The fact that the processus vaginalis forms well prior to testicular descent and has extended far into the scrotum before this event, gives no support to the thesis put forward by Curling and by Moscowitz among others, that the descending testis 'drags' the peritoneum with it to form the processus vaginalis. For such a thesis to be acceptable, the formation of the processus vaginalis must be closely linked both in time and extent with testicular descent. In man, a reasonably close linkage exists between these events, so that such a mode of formation of the processus vaginalis is perhaps not unreasonable, but in the pig the two processes are so widely separated in both time and extent that no direct mechanical linkage can possibly be entertained. The presence of actively growing primitive type coelomic epithelial cells at the point of extension of the processus vaginalis into the gubernaculum testis, whilst elsewhere the coelomic epithelial cells are of a specialized flattened form points to a simple invasion process taking place. Such an active coelomic invasion of the gubernacular mesenchyme offers a rational explanation of the mode of extension of the peritoneal cavity to form the processus vaginalis in the male and, also, the formation of the canal of Nuck in the female which was quite outside the range of the Curling-Moscowitz theory.

Just as the formation of the processus vaginalis is less intimately linked with the actual descent of the testis in the pig than man, so also is the development of the cremaster muscle. In man the medial and lateral cremaster muscles are initially formed quite early in embryonic development but do not extend beyond the inguinal canal until shortly before the testis descends. In the pig, however, as the processus vaginalis extends distalwards so the cremaster muscle differentiates *within the gubernacular mesenchyme* along the root of the mesentery of the plica gubernaculi but in the pars vaginalis gubernaculi. It develops as a single lateral bundle extending only so far distalwards as the processus vaginalis, the two developing both spatially and temporally in unison and well in advance of testicular descent. Such developmental features amply illustrate that the cremaster muscle, in the pig at least, does not consist of muscle fibres dragged off the abdominal wall by the descending testis as was postulated by Cloquet, Carus and Moscowitz. Furthermore, the early distally directed differentiation of the muscle towards the scrotum in the pig does not permit consideration of an 'inverted' cremaster muscle running from an inguinal origin to the abdominal testis and capable of hauling the testis down into the scrotum as Seiler, Curling, Lockwood and many other authors have postulated as taking place in man and certain other mammals. If the cremaster muscle were to contract in a pig foetus, the testis would be elevated on its plica gubernaculi rather than descend, an action comparable with that of the adult cremaster muscle.

The mesenchymatous nature of the gubernaculum testis has been described in part by previous authors, but details of its complete separation from the scrotal wall have been neglected. It is unlikely that this separation has not been seen, probably having been dismissed as an artefact, a distal attachment being essential if the traditional contractile role is assigned to the gubernaculum testis. That the separation is not an artefact is quite obvious from histological study. The gubernaculum testis is initially continuous with the remaining mesenchyme of the scrotum and of the abdominal wall, but when this differentiates the gubernaculum testis becomes distinct as a mesenchymatous structure and a separation zone forms. This zone remains as loose tissue so long as the gubernaculum remains small, but, with distal expansion due to changes in the ground substance, so forming a globular mass within the scrotal swelling, separation of the gubernaculum testis from the scrotal wall becomes complete. A factor involved in the separation process is the differentiation of the scrotal wall, which does not change shape directly with the changes occurring in the gubernaculum.

Consequent upon these changes and the cessation of growth of the plica gubernaculi relative to the surrounding structures, the gubernacular tip becomes withdrawn from the scrotal floor. It must be emphasized however that this withdrawal, of up to 5 mm., is much more marked in the pig, which has a perineal scrotum, than in the sheep or man in which the scrotum is dependent. Due to the perineal position of the pig scrotum, the gubernaculum testis is falciform, whereas that of the sheep or man is more nearly straight. Increase in thickness of the pig gubernaculum around its curve tends to carry the distal portion of the gubernaculum forwards out of the perineal scrotum. The gubernaculum testis of sheep or man is only affected by differential growth between itself and the surrounding tissues and only rises some 1–2 mm. from the scrotal floor.

Some shortening of the combined plica et pars infravaginalis gubernaculi is obviously essential if the testis is to pass into the scrotum, although no active contractile shortening occurs, for the inguinal canal and scrotum are completely filled by the gubernaculum testis, leaving no room for the testis. Shortening however is shown to be a relative factor due to a cessation of growth in these parts of the gubernaculum, while the surrounding tissues (including the pars vaginalis gubernaculi) continue to grow. Thus with relative gubernacular shortening there will be progressive retraction of the gubernacular tip from the floor of the scrotum if the testis does not descend. However, lengthening of the pars vaginalis gubernaculi will permit the gubernacular tip to remain close to the floor of the scrotum and the testis to descend. This movement is facilitated by the plica gubernaculi taking on a conical form, surmounted by the testis, and dilating the scrotum and, in particular, the inguinal canal. Such a mechanical arrangement permits the gubernaculum testis and the testis to slide distalwards to fill the space in the scrotum created by the cessation of gubernacular growth.

The movement of the testis through the inguinal canal is rapid, for although many foetuses were examined about the time of testicular descent, in only one specimen could the testis be considered in transit. In this specimen the testis could not be pushed onwards through the inguinal canal, the vas deferens being too short to permit further testicular descent. It is suggested that, in view of the rapidity of testicular descent, some motivating force such as increased intra-abdominal pressure due to intra-uterine respiratory movements, may initiate passage of the testis through the inguinal canal once the canal has been dilated by the gubernaculum testis. It is submitted, however, that such a motivating force is of little significance, for the mechanical arrangement of the gubernacular apparatus described is sufficient to precipitate testicular descent.

Clearly, this testicular translation must be preceded by sufficient growth in length of the vas deferens and testicular vessels. The vas deferens is initially too short to permit testicular descent, and in the single case of arrested descent described herein, the limiting factor was the length of the vas deferens. The growth in length of the vas deferens and in particular of the cauda epididymis is appreciable from the time of testicular descent until birth. The gubernaculum testis forms a ready medium into which the cauda epididymis can expand. This expansion of the epididymis stimulates testicular descent, but mensuration shows no actual shortening of the distance between the lower pole of the testis and the distal extremity of the processus vaginalis or the floor of the scrotum, once the testis has passed through the inguinal canal. Thus, once the testis has passed through the inguinal canal no absolute testicular descent takes place, though relative to the animal as a whole such a descent is obvious. The final stages are thus brought about, not by an intrinsic function of the plica gubernaculi but by simple lengthening of the pars vaginalis gubernaculi and of the vas deferens and testicular vessels, associated with lengthening of the distance between the external inguinal ring and the floor of the scrotum. Under normal conditions the gubernaculum testis does not remain as a well-organized residual band as is often asserted but changes into the connective tissues in the neighbourhood of the testis. The thin sheath of mesenchyme which remains from the partes vaginalis et infravaginalis gubernaculi, and which surrounds the processus vaginalis and its contents, becomes the fascial coverings of the testis and cord. In the pig, this covering is not readily divisible into three layers as it is in man. The cremaster muscle of the pig is a single fasciculus representing only the lateral cremaster muscle. Hence, instead of medial and lateral cremasteric fibres interdigitating around the testis and cord, with deep and superficial fascial covering layers as in man, a single fasciculus runs distalwards along the line of the mesorchium, with the vessels and vas deferens. Except along this line the spermatic fascia is simple and not split into internal and external layers by the cremaster muscle. As in man (Backhouse & Butler, 1955) the external spermatic fascia develops only a *fascial* continuity with the margins of the external ring after the development of the processus vaginalis, and after the testis has descended and is not formed by ‘dragging down’ the external oblique aponeurosis. Fibrous tissue forms in the pars vaginalis gubernaculi and becomes joined proximally with the external inguinal ring.

Testicular descent may be divided into three phases; two of these are slow processes dependent upon the relative growth of various organs and are connected by a rapid propulsion of the testis through the inguinal canal.

1. When the testis first develops in the abdomen, it is separated from the future internal inguinal ring by the large mesonephros. As a result of testicular growth and mesonephric degeneration the caudal pole of the testis comes to lie very close to the internal inguinal ring.

2. In foetuses of 190–200 mm. C.R. length, the testis passes through the inguinal-canal to a position high in the groin just outside the external inguinal ring, facilitated by changes in the gubernaculum testis and growth in length of the vas deferens and testicular vessels. The available evidence suggests that this passage is very rapid and it is possible that the motivating force may be intra-uterine respiratory movement, though dilatation of the inguinal canal by the gubernaculum testis is the main factor involved.

3. Final descent is partly apparent and partly real. The apparent further descent is brought about by the caudal expansion of the cauda epididymis into the plica gubernaculi to approach the scrotal floor. Any real descent is a result of growth in length of the scrotum, the vas deferens and testicular vessels and the pars vaginalis gubernaculi (including the cremaster muscle).

SUMMARY

The gubernaculum testis is described in the domestic pig (*Sus scropha*) from its first appearance in the early embryo until eventual change to the adult form. Its three component parts are named and defined. It is a simple mesenchymatous structure, around which the body wall muscles and fasciae differentiate, and into which the processus vaginalis grows and the cremaster muscle differentiates. No muscular tissue, which could bring about testicular descent, extends from the distal end of the gubernaculum to the abdominal testis. Towards the time of testicular descent, the distal end of the gubernaculum testis comes to lie free in the scrotum and the gubernacular ground substance is greatly increased in amount; the scrotum and inguinal canal thus become dilated in advance of testicular descent. Passage of the testis through the inguinal canal is rapid and is preceded by lengthening of the vas deferens and testicular vessels. This movement represents the only true descent of the testis. Further descent to the floor of the scrotum is due to lengthening of the structures of the testicular cord and their coverings and the invasion of the distal portion of the gubernaculum by the growing cauda epididymis. After testicular descent the remains of the gubernaculum testis are converted into the fascial coverings of the testis and cord and the connective tissue around the cauda epididymis.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Section of a 38 mm. c.r. length pig embryo showing the gubernaculum testis in the region of the inguinal canal and showing the plica gubernaculi extending from the surface of the mesonephros to the anterior abdominal wall, the distal 0·1 mm. being within the processus vaginalis.

Fig. 2. Section of a 45 mm. c.r. length pig foetus showing the plica gubernaculi extending from the large mesonephros into the gubernaculum of the inguinal canal region. The processus vaginalis extends some 0·2 mm. into this gubernacular mesenchyme and the early differentiating M. cremaster can also be seen.

Plate 2

Fig. 3. Section of a 45 mm. c.r. length pig foetus showing the mesenchyme of the scrotal region. The gubernacular mesenchyme can only be recognized at this stage as a slightly denser region.

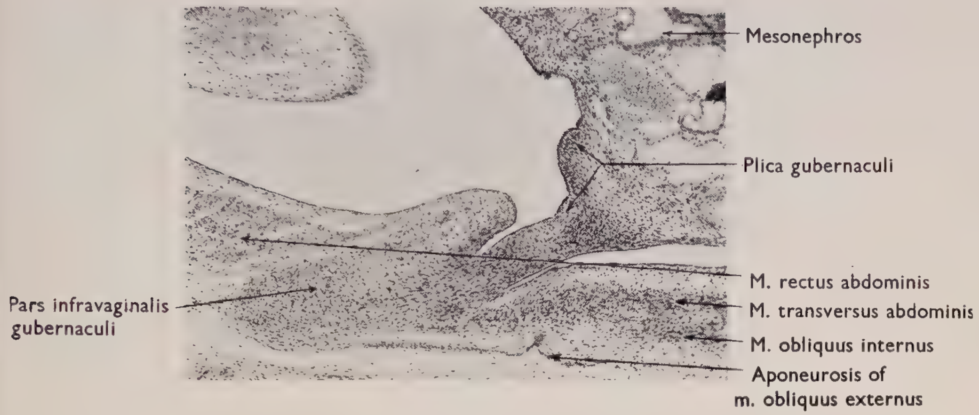
Fig. 4. Section of the inguinal region of an 85 mm. c.r. length pig foetus showing the gubernacular mesenchyme containing the cremaster muscle and the processus vaginalis.

Plate 3

Fig. 5. The sectioned testis and gubernaculum testis of a 165 mm. c.r. length sheep foetus; the structures have been divided around the inguinal region but the distal portion has merely been lifted from the scrotal sac.

Fig. 6. Section of testis and gubernaculum testis of a 310 mm. c.r. length pig foetus. The testis has descended into the scrotum and marked growth of the cauda epididymis within plica gubernaculi can be seen.

Fig. 7. Transverse section through the gubernaculum testis of a 290 mm. pig foetus showing the plica gubernaculi and the now well-differentiated pars vaginalis gubernaculi, containing the M. cremaster.



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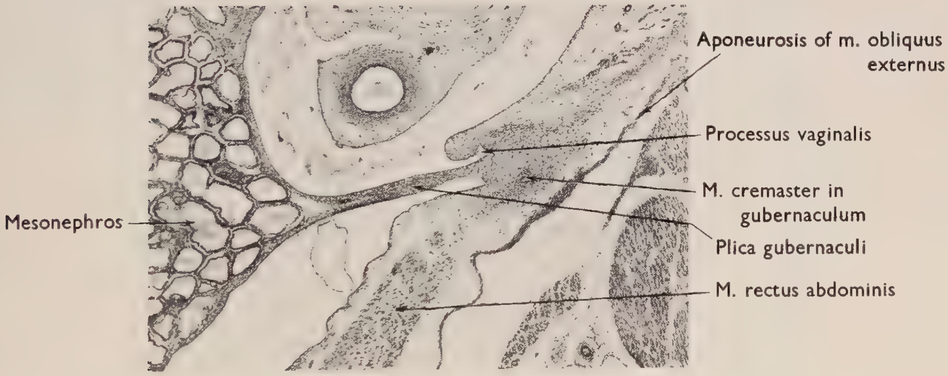

2

BACKHOUSE AND BUTLER—THE GUBERNACULUM TESTIS OF THE PIG (*SUS SCROPHA*)

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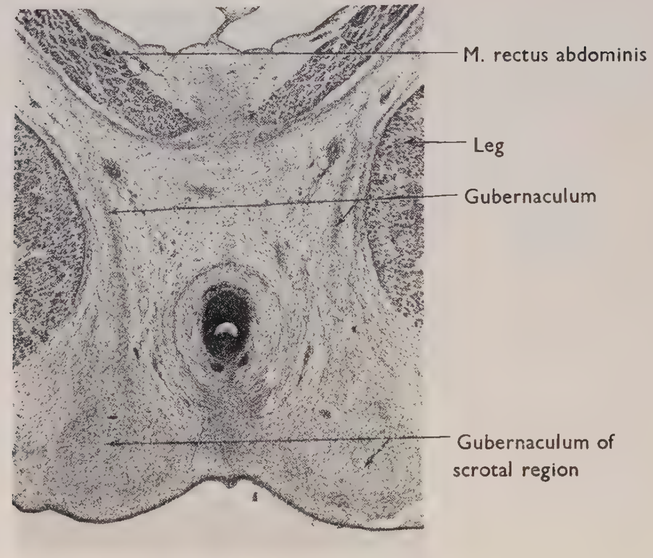

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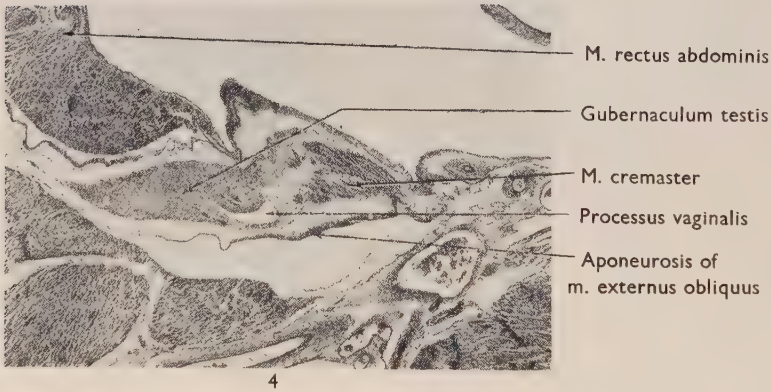

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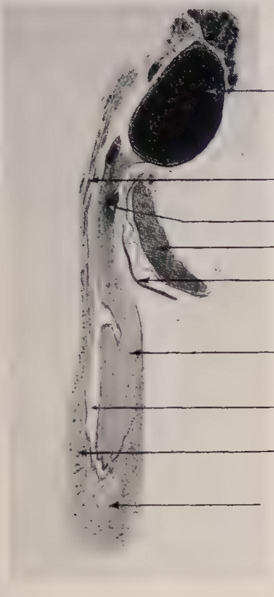
BACKHOUSE AND BUTLER—THE GUBERNACULUM TESTIS OF THE PIG (*SUS SCROPHA*)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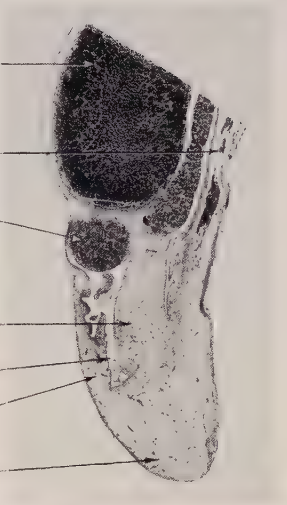

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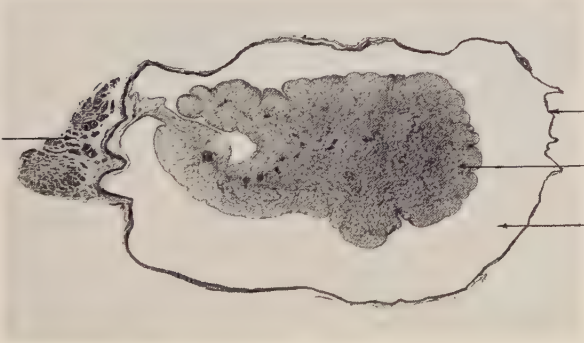

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BACKHOUSE AND BUTLER—THE GUBERNACULUM TESTIS OF THE PIG (*SUS SCROPHA*)

DEVELOPMENT OF THE TERMINAL URETHRA AND PREPUCE IN THE DOG

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There is general agreement on the development of the proximal part of the penile urethra in man and in other mammals while opinions vary on the mode of formation of its distal part. Felix (1912) considered that in man the latter is formed by the fusion of the urethral folds subsequent to the dissolution of the entodermal urethral plate. He believed that during this fusion surface ectoderm is excluded from contributing to the lining of the distal part of the penile urethra. Thus, in his view, the entire urethra is entodermal in origin. Wood Jones (1910) thought that the urethra within the glans penis is the result of canalization of a surface ectodermal in growth into the substance of the developing glans penis. Siddiqi (1937) found that the major part of the penile urethra in the spermophile is developed both from ectodermal and entodermal sources while the urethra within the glans is derived entirely from an infolding of surface ectoderm. Barnstein & Mossman (1938) observed that the penile urethra in the red squirrel is developed from canalization of the urethral plate which they believe to have originated from an ingrowth of ectoderm beginning in front of the cloacal opening and extending to the tip of the phallus. They concede, however, that the proximal portion of the plate may be derived from entoderm of the urogenital sinus. Williams (1952) considered that the urethra within the glans penis in man is the result of fusion of the urethral folds subsequent to the canalization of the urethral plate. Unlike Felix, he contends that surface ectoderm also enters into the formation of the glandar urethra. More recently, Glenister (1954) has observed that the distal part of the glandar urethra in man is formed by a lamellar ingrowth of surface epithelium which gives rise to the urethra as far back as the valve of Guerin. The remainder of the glandar urethra, according to him, is divisible into two portions, a proximal part derived solely from the entodermal urethral plate and a distal part which is partly entodermal and partly ectodermal in origin.

Differences of opinion also exist on the mode of formation of the prepuce. Wood Jones (1910) and Johnson (1920) maintained that it is formed as a result of canalization of an epithelial ingrowth into the mesoderm of the distal part of the phallus. Hunter (1935) considered that a preputial fold grows over the naked glans penis and fusion occurs between the epithelium lining the inner surface of the fold and that covering the glans penis. Subsequently, a breakdown of cells occurs within the fused epithelial layers and liberates the glans penis from the prepuce. Glenister (1956*b*) describes the prepuce as being formed by a combination of epithelial ingrowth and fold formation.

The site of the urethral plate in the human embryo appears to correspond to that of the os penis of some mammals. Embryos of a mammal possessing a prominent os penis were therefore chosen for the present investigation to find out whether there is in fact a transformation of the 'epithelial' urethral plate into the primordium of the os penis. Our observations revealed that no such transformation of the 'epithelial' cells of the urethral plate into the mesodermal tissue of the os penis takes place in normal development. However, the mode of development of the terminal urethral and prepuce in the dog were found to provide sufficient interest as to justify the present communication.

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MATERIAL AND METHODS

The caudal parts of a series of twelve dog embryos ranging from 11 to 170 mm. C.R. length were sectioned serially in the transverse, sagittal and coronal planes at 10μ thickness. Most of the sections were stained by haemotoxylin and eosin while the periodic acid Schiff technique was employed to demonstrate the basement membrane in a few selected sections. The sex of the embryos was determined by using the criteria adopted by Gillman (1948).

OBSERVATIONS

11 mm. C.R. length embryo. The distal end of the urethral plate is situated about 70 μ behind the tip of the phallus and continues proximally for a length of 90 μ before merging with the entodermal wall of the anterior portion of the cloaca. The basement membrane lining the roof and side walls of the urethral plate is continuous with the basement membrane beneath the surface ectoderm of the phallus except in the area which is directly beneath the urethral plate (Pl. 1, figs. 1, 2). Transverse sections through the urogenital part of the cloaca likewise show a continuity of the basement membrane, which is beneath the entodermal lining of the cloaca, with that lying deep to the surface ectoderm on the ventro-lateral and dorsal surfaces of the phallus. The cloacal membrane which forms the floor of the cloaca is about 4–7 cells thick along its entire length. There is a higher number of mitotic figures in the entoderm of the anterior cloacal region than in the urethral plate. Mitosis is even less evident in the ectoderm adjacent to the plate.

15 mm. C.R. length embryo. The urethral plate is now 760 μ in length and has reached to within 40 μ of the tip of the phallus. The proximal part of the plate is Y-shaped in transverse section and shows discrete clefts which coalesce proximally to become continuous with the phallic portion of the urogenital sinus. A groove runs longitudinally along the ventral surface of the phallus immediately beneath the urethral plate.

In another embryo of this size, sectioned sagittally, the urorectal septum separating the urogenital sinus from the rectum is clearly seen (Pl. 1, fig. 3). The anal membrane is thin and shows signs of imminent rupture, whereas the urogenital membrane retains the same thickness as in the 11 mm. embryo. The cavity of the urogenital sinus is seen to extend into the proximal third of the free part of the phallus while the urethral plate is confined to its distal two thirds.

25 mm. C.R. length embryo. The under surface of the phallus in relation to the urethral plate is grooved and part of the surface ectoderm covering the plate appears to have been lost. The urethral plate, when traced proximally, begins to widen and the groove becomes progressively deeper resulting in loss of surface cells in its depth. More proximally, the edges of the groove converge and the urethral plate in this region shows considerable enlargement in its deeper portions. Epithelial bridges are present across the groove converting it into two or three compartments. These bridges have the same histological appearance as the cells of the urethral plate. Close to the base of the phallus the superficial skin folds fuse beneath the epithelial bridges resulting in the formation of the median raphe which forms an elevated ridge. However, the surface epithelium appears to have been completely excluded from contributing to any portion of the urethral wall. There is as yet no indication of the formation of the prepuce.

In another 25 mm. embryo, cut sagittally, the phallic part of the urogenital sinus is still shut off from the exterior by a thin urogenital membrane which now resembles the thin anal membrane seen in the 15 mm. embryo.

36 *mm.* c.r. *length embryo.* The entire free portion of the phallus constitutes the glans penis which is now demarcated from the rest of the phallus by the developing prepuce. The formation of the prepuce is seen as a mesodermal fold growing into the thickened epithelial layers covering the glans penis. This mesodermal outgrowth carries with it the germinal layer of the surface ectoderm which as a result becomes reduplicated (Pl. 1, fig. 5). The so-called glandular lamella is thus formed between the germinal layer covering the glans penis and that lining the inner surface of the mesodermal outgrowth (Pl. 1, fig. 5). The presence of a large number of mitotic figures taken in conjunction with the great degree of vascularity which characterizes the apex of the preputial mesoderm provides the histological evidence for the proliferative activity in this region. By contrast, the glandar lamella shows only occasional mitosis. The basement membrane lying beneath the surface ectoderm of the proximal part of the phallus extends almost to the apex of the preputial mesoderm; it is indistinct or absent over the surfaces of the glandar lamella. In a 29 mm. embryo cut sagittally, the preputial area presents a picture essentially similar to that seen in the 36 mm. embryo except that the vascularity within the preputial mesoderm is less obvious and the basement membrane around the glandar lamella is uninterrupted (Pl. 1, fig. 4).

The relationship of the urethral plate in the 36 mm. embryo is confined to the ventral surface of the glans penis and runs through its entire length to reach the surface ectoderm at the apex of the glans. The surface ectoderm immediately dorsal to this area of contact is grooved as a result of disintegration of some of the surface epithelial cells.

43 *mm.* c.r. *length embryo.* As in the preceding stage the entire free portion of the phallus constitutes the glans penis. The prepuce itself now extends over the proximal half of the glans and the glandar lamella appears as an epithelial ingrowth; it is longer and extends more proximally on the dorsal surface than on its ventral surface. Over the distal half of the glans penis the surface epithelium shows considerable proliferation except at the apex of the glans where the urethral plate has made contact with it. Here the surface epithelium shows degenerative changes resulting in an epithelial pit or groove.

50 *mm.* c.r. *length embryo.* The epithelial pit or groove situated at the apex of the phallus is now quite distinct. It is open ventrally and its roof and side walls are

formed by surface ectoderm. When traced proximally the epithelial pit narrows and its place is eventually taken up by the distal end of the urethral plate. Whether or not the epithelial pit had eroded through the surface epithelial layers into the distal end of the urethral plate could not be determined. The presence of cavernous tissue dorsal to the proximal end of the epithelial pit perhaps favours the view that the pit has indeed extended into the distal extremity of the plate.

Sections taken at appropriate intervals from the epithelial pit towards the base of the glans show the following significant changes: (*a*) the grooved urethral plate lies along the ventral surface of the distal end of the glans penis where there is no attempt at formation of the urethra; (*b*) fusion of the urethral folds has proceeded in such a way as to incorporate a part of the surface ectoderm into the side walls and floor of the urethra; (*c*) the mesoderm of the urethral folds is directly continuous with that of the prepuce; (*d*) the separation of the urethral wall from the ventral portion of the glandar lamella has been brought about by the invasion of the mesoderm of the glans towards the under surface of the urethra; (*e*) the urethral plate which has hitherto remained compact on the roof of the urethra now has opened out to form the roof and side walls of the urethra. The cells of the urethral plate are easily recognizable throughout the entire length of the glans penis. They differ histologically from surface ectodermal cells which partly contribute to the urethral wall.

62 *mm. c.r. length embryo.* The epithelial pit is essentially similar to that observed in the 50 mm. embryo. The floor of the proximal part of the pit has now been sealed off by a projecting tongue of epithelial cells from the anterior margin of the fused urethral folds. The fusion of these folds has almost reached the distal extremity of the glans penis resulting in the formation of the perineal raphe. The urethral plate forms the roof of the glandar urethra for about its distal fourth; more proximally the plate opens out to form the roof and side walls of the glandar urethra. The surface ectodermal cells, which partly contribute to the lining of the glandar urethra, are distinguishable from those of the urethral plate over the distal half of the glans penis. In the proximal half of the glandar urethra, however, these morphological differences disappear with the result that the urethral lining presents a uniform appearance.

84 *mm. c.r. length embryo.* The prepuce now extends to the tip glans penis where the terminal epithelial pit is situated. The mesoderm of the urethral folds has invaded the lateral lips of the pit and, in fact, extends into the tongue-like epithelial projection which, in the 62 mm. embryo, formed the floor of the proximal part of the pit. Continuous with the terminal pit is the distal part of the glandar urethra, the lumen of which is made considerably larger by disintegration of the central cells of the swollen urethral plate.

The distal half of the glandar urethra is now lined by a stratified epithelium which, in the floor and the adjacent portions of the side walls consists of pale clear-looking cells with distinct cell outlines; in the remaining parts of the side walls and in the roof of this part of the urethra the epithelium is formed by cells which stain intensely with eosin and with ill-defined cell boundaries between them. The lighter staining cells resemble the surface ectodermal cells while the remainder appear to be derived from the urethral plate. There are no recognizable morphological differences in the cells lining the roof and floor of the proximal half of the glandar urethra.

110 *mm.* C.R. *length embryo.* The epithelial pit has become deeper by further extension of the urethral fold mesoderm into the lateral lips guarding the pit. Proximally, the two folds guarding the pit fuse to form the floor and lateral walls of the preputial opening. The roof of the preputial opening is formed by the original roof of the epithelial pit and by the mesoderm of the prepuce which has invaded the epithelial roof. The preputial opening, which was formed by disintegration of the surface epithelial cells, is directly continuous with the terminal orifice of the glandar urethra.

There is no morphological distinction between the cells lining the roof and floor of the distal part of the glandar urethra, but the number of epithelial layers forming the wall of the urethra has become less than in the preceding stage. The proximal part of the glandar urethra also shows a reduction in the stratification of its epithelial layers. The prepuce now extends beyond the tip of the glans penis. There are bud-like protrusions emerging from the outer surface of the glandar lamella, while the inner surface of the lamella adjacent to the erectile tissue of the glans penis remains smooth.

170 *mm.* C.R. *length embryo.* The epithelium in the distal part of the glandar urethra shows a definite change towards the stratified squamous type while the epithelium in the proximal part of the glandar urethra continues to be of the embryonic type as in the 110 mm. embryo. The glandar lamella shows degenerative changes which proceed from the distal end of the glans penis towards its base. The mesodermal condensation representing the primordium of the os penis shows enlargement and separation of its component cells; these changes possibly herald the precartilaginous phase in the development of the os penis.

DISCUSSION AND CONCLUSIONS

Urethral plate. The urethral plate is described by Glenister (1954, 1956*a*) as a lamellar outgrowth from the anterior wall of the entodermal cloaca. The present observations support this conclusion. Those who hold the view that the urethral plate is an ectodermal derivative (Barnstein & Mossman, 1938) may have been misled by the fact that in transverse sections of the phallus, there is no distinct demarcation between the surface ectodermal cells lying ventral to the urethral plate and the plate itself. Thus, the basement membrane lining the roof and side walls of the urethral plate is continuous with the basement membrane lying deep to the surface ectoderm on the dorsal and lateral surfaces of the phallus. In our view, this continuity does not warrant the conclusion of an ectodermal origin for the urethral plate. It seems likely that a basement membrane is usually formed between an epithelium and its underlying mesodermal tissue rather than between 'epithelial' cells derived from different germ layers. This may account for the absence of the membrane between the surface ectoderm and the underlying urethral plate (Pl. 1, figs. 1, 2). It is also possible that conditions are less stable in the region of the plate so as to prevent the formation of a basement membrane.

The larger proportion of mitotic figures found in the anterior wall of the entodermal cloaca than elsewhere in this region lends additional support to the view that the urethral plate is, in fact, of entodermal origin. The epithelial nature of the urethral plate must be emphasized; transformation of the plate cells into mesodermal tissue seems most unlikely. There seems, indeed, to be a close resemblance between the urethral plate and the epithelial vaginal plate each of which canalizes and eventually forms the lumen of a tube.

Glandar urethra. The glandar urethra in the dog is formed by the progressive fusion of the urethral folds after the superficial epithelium covering the under surface of the urethral plate has been cast off. The fusion of the urethral folds proceeds from the base of the phallus towards its apex and results in the formation of the perineal raphe. The fusion of the folds occurs in such a way that surface epithelium is incorporated into the floor of the urethra in the proximal part of the glans while surface epithelium contributes to the floor and side walls of the glandar urethra in its distal part. The rest of the lining of the glandar urethra is contributed by the urethral plate. An important feature during the process of fusion is that the mesoderm within the urethral folds breaks through the surface epithelium covering the area of the folds where fusion occurs. This break-through is significant in that it tends to separate surface epithelial layers which form normal skin from the rest of the ectoderm which is potentially different and which contributes to a portion of the wall of the urethra. Examples of equivalent segregation of groups of cells which are potentially different are recorded by Streeter (1942).

The views outlined above on the development of the glandar urethra in the dog concur with those of Williams (1952) but differ from the findings of Glenister (1954) who claims that in man the distal part of the glandar urethra, up to the valve of Guerin, is formed by an ingrowth of surface epithelium. At no stage in the development of the glandar urethra in the dog was any ingrowth of surface epithelium seen.

The formation of the terminal orifice of the urethra like that of the opening of the preputial sac results from loss of surface epithelium covering the apex of the phallus. It is possible, however, that this process of erosion might have extended into the distal tip of the urethral plate. Such a concept of the formation of the preputial and urethral openings provides a satisfactory explanation of certain findings associated with hypospadias in man.

During the early stages in the development of the glandar urethra, the cells lining the roof are morphologically distinct from those lining the floor of the urethra. These differences disappear, however, so that by the 110 mm. stage they are noticeable only in the distal part of the glandar urethra. At the end of foetal life, there are no recognizable differences between the roof and floor cells even in the distal part. The lining of the glandar urethra is then recognizable as stratified squamous epithelium. It is difficult to ascertain how these changes are brought about. They are more likely to result from local metaplasia than from a spread of stratified squamous epithelium from the surface ectoderm. This view of local metaplasia receives support from the fact that different types of epithelia were found on the same surface of the obstructing membrane in one of the cases of primary cryptomenorrhoea reported by Kanagasuntheram & Dassanayake (1958).

Prepuce. The prepuce in the dog begins to develop as a mesodermal outgrowth into the thickness of the epithelial layers covering the distal part of the phallus which is to form the future glans penis. The growing mesodermal process carries with it the germinal layer of surface ectoderm which as a result becomes reduplicated. There are, in fact, three layers of germinal epithelium, two of which line the inner and the outer surfaces of the mesodermal fold while the third or innermost layer forms the immediate covering for the erectile tissue of the glans penis. This so-called glandar lamella is formed between the erectile tissue of the glans penis and the inner surface of the mesodermal fold. In the dog the splitting of the glandar lamella and the consequent separation of the prepuce from the glans is not accomplished even at the end of foetal life although degenerative changes have already set in within the lamellar epithelium. This contrasts with the observations of Gairdner (1949) and Glenister (1956*b*) that in man a breakdown of the glandar lamella has commenced during foetal life. The absence of 'epithelial pearl' formation in the dog is in accord with similar findings by Glenister (1956*a*) in the pig.

The mesodermal outgrowth which forms the anlage of the prepuce produces a separation of the deeper layers of the surface ectoderm covering the glandar region of the phallus from those forming the more superficial layers. It would therefore appear that the glandar lamella, during its genesis, incorporates within it the deeper layers of surface ectoderm. The growth of the mesoderm and the consequent separation of the epithelial layers into distinct laminae proceeds from the base of the glans penis towards its distal end where the preputial opening and the external urethral orifice have both been formed as a result of a loss of surface epithelium covering the apex of the glans penis. The growth of the mesoderm into the epithelial layers causes a thinning of the surface ectoderm in the basal portion of the glans where the prepuce is developing while the ectoderm covering the remaining portion of the glans continues to remain thick. The significance of the separation between the superficial and deeper layers of the surface ectoderm becomes clear in the light of a similar separation between potentially different ectodermal layers during the process of fusion of the urethral folds.

The invasion of mesoderm into the surface ectoderm is closely followed by the differentiation of the urethral folds in the region of the glans penis. There is also a continuity between the mesoderm of the prepuce and that of the folds. Further, cases of hypospadias in man show an absence of the inferior segment of the prepuce (Paul & Kanagasuntheram, 1956). From these observations it might be inferred that the urethral folds, in fact, contribute to the inferior segment of the prepuce.

The development of the prepuce in the dog, as outlined in this paper, differs from views already expressed on this subject for other mammals. Wood Jones (1910) and Johnson (1920) believed that the prepuce resulted from an ingrowth of a glandar lamella. If this view is correct, one would expect the glandar lamella to commence at the apex of the phallus and work its way towards the base of the glans penis. Such a lamellar ingrowth is not seen in sections of the developing prepuce in the 29 mm. and the 36 mm. embryos. On the contrary, the formation of the prepuce proceeds from the base of the glans towards its apex. The concept of Berry Hart (1908) and Hunter (1935) that the growth of a preputial fold over the naked glans would explain the formation of the prepuce, appears to be incorrect. If the preputial fold with its covering epithelium moved forwards over the epithelium of the naked glans, the combined epithelial layers which give rise to the glandar lamella should be thicker than the epithelium over the naked area of the glans. But observation shows (Pl. 1, fig. 5) that the glandar lamella is much thinner than the surface ectoderm covering the distal part of the glans which is still free of the developing prepuce. For these reasons a combination of epithelial ingrowth and preputial fold formation as propounded by Glenister (1956*b*) would be equally inadequate to explain the formation of the prepuce. But it must be admitted that Glenister's view on the formation of the preputial fold is totally different to that envisaged by earlier authors. According to him, 'histological appearances and reconstructions suggest that the mesenchyme situated between the surface epithelium covering the so-said fold and the glandar lamella proliferates and draws the superficial origin of the glandar lamella with it. In this way the margin of the fold, which is continuous with the glandar lamella and thus not free, is pushed distally till it reaches the summit of the glans'. However, it is not clear how, during this process, the advancing glandar lamella escapes from incorporating within it some of the cells belonging to the deeper layers of the surface ectoderm covering the glans. The present observation of a mesodermal fold carrying with it the germinal layer and growing into the thickness of the epithelial layers and the resultant lamellar formation, do in fact provide a satisfactory explanation of the various changes observed during the course of development of the prepuce.

As regards the ingrowth of the glandar lamella, it may be stated that the histological picture in the 36 mm. embryo is one of slow proliferation with few mitotic figures within the lamella. In the face of the evidence found in the 29 mm. embryo, in which the basement membrane is seen to continue round the glandar lamella (Pl. 1, fig. 4) and in which the lamellar cells hardly exhibit any mitosis, it may be argued that there is no ingrowth of the lamella at this early stage of development of the prepuce but merely a forward growth of the mesodermal fold as mentioned earlier. But, once the lamella has been formed, it elongates to keep pace with the interstitial growth of the glans. Such an interpretation fits in closely with the slow proliferation within the lamella. This mode of elongation of the lamella must, however, be distinguished from an active growth of the lamella into the substance of the glans.

The degenerative changes within the lamella and, presumably, the glando-preputial separation proceed from the apex of the glans penis towards its base. But these changes cannot be viewed as confirmatory evidence that the glandar lamella is an ingrowth in which the deeper portions retain their proliferative activity for a longer period. An entirely different interpretation may be attached to these degenerative changes, i.e. whenever a solid plate of epithelial cells begin to canalize, the process is seen to commence from the side of the plate which is nearest a cavity.

SUMMARY

1. The development of the urethra within the glans penis and of the prepuce has been followed in a series of dog embryos ranging from 11 to 170 mm. C.R. length.

2. The significance of the urethral plate and the relative contributions of ectoderm and entoderm to the lining of the glandar urethra has been discussed.

3. The prepuce begins to develop as a mesodermal outgrowth into the thickened epithelial layers covering the glans penis.

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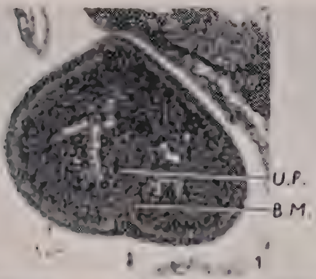

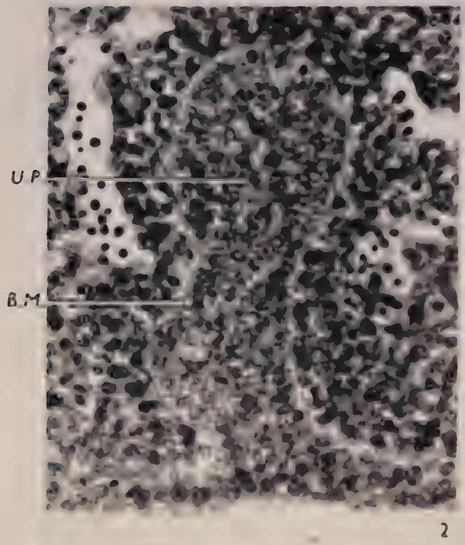

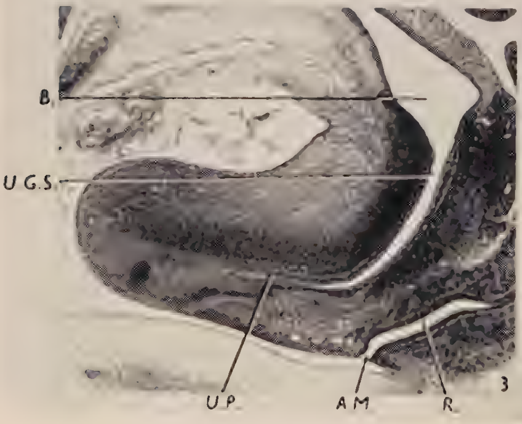

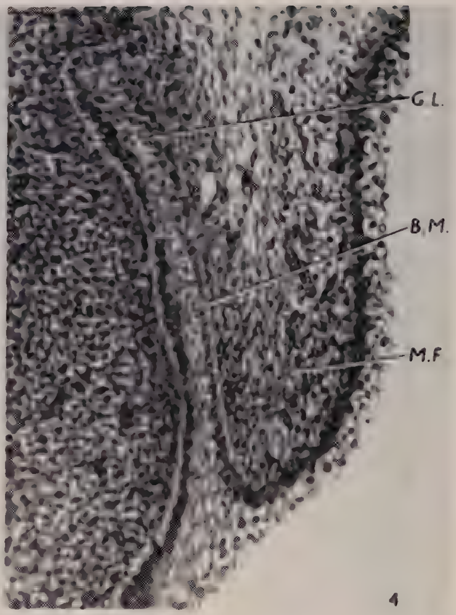

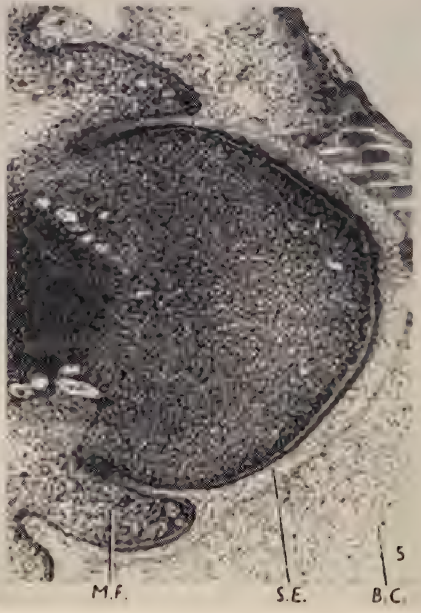

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EXPLANATION OF PLATE

List of abbreviations

A.M.	Anal membrane.	*M.F.*	Mesodermal fold of prepuce.
B.	Bladder.	*S.E.*	Surface ectoderm.
B.C.	Blood clot over glans penis.	*U.G.S.*	Urogenital sinus.
B.M.	Basement membrane.	*U.P.*	Urethral plate.
G.L.	Glandar lamella.	*R.*	Rectum.

Fig. 1. Transverse section through the phallus of the 11 mm. dog embryo showing the continuity of the basement membrane around the urethral plate with that beneath the surface ectoderm of the phallus. × 84.

Fig. 2. High power view of the urethral plate and the basement membrane surrounding it. × 375.

Fig. 3. Sagittal section through the caudal part of a 15 mm. embryo showing the thin anal membrane. Note the absence of a urogenital membrane at this stage. × 45.

Fig. 4. Sagittal section through the phallus of the 29 mm. embryo to show the continuity of the basement membrane round the glandar lamella. × 210.

Fig. 5. Coronal section through the phallus of the 36 mm. embryo to show the formation of the prepuce. Note the difference in thickness of surface ectoderm over the preputial fold of mesoderm and that covering the distal end of the glans penis. × 84.

A HISTOCHEMICAL STUDY OF THE ACCESSORY GENITAL GLANDS OF THE BOAR

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INTRODUCTION

Review of the relevant literature suggested that, particularly from the histochemical point of view, the accessory genital organs of the boar had not been studied exhaustively and, as a previous study of these organs in the ram (Aitken, 1955, 1959) had shown that species differences may be considerable, it was decided to re-examine them with particular reference to histochemistry.

In the ram the prostate is exclusively disseminate in type, consisting of numerous small tubular glands in the subepithelial connective tissue of the pelvic urethra, and a previous study (Aitken, 1959) has shown that these are functionally similar to the bulbourethral gland and in fact may be regarded with some justification as forming accessory bulbourethral tissue. Similar though less numerous glands are found occasionally in man (Lichtenberg, 1906; Johnson, 1920). In the boar the prostate is composed of compact and disseminate parts. McKenzie, Miller & Bauguess (1938) thought that the latter could be subdivided into two functionally distinct parts, but did not characterize these histochemically. They came to the conclusion, as the result of their studies on fractionated boar semen, however, that discharge of the secretion of the bulbourethral gland began even before the penis entered the vagina and that this secretion, together with some prostatic secretion, acted as a lubricant. They did not determine which part of the prostate was involved in the initial secretion, however, and in view of the similarity between ram disseminate prostate and bulbourethral gland, it seemed possible that part at least of the disseminata in the boar might resemble the bulbourethral gland and that this part alone was involved in the initial secretion. This is of particular interest in view of the fact that the extremely viscid nature of bulbourethral secretion suggests a function other than lubrication and it may be that it is only useful for this purpose when mixed with other secretions.

MATERIALS AND METHODS

Specimens from adult boars were collected at the local abattoir. Usually the slaughter of the animal was observed and the gland dissected out immediately afterwards and transported without delay to the laboratory or, if freeze-drying or chilled fixatives were to be used, selected portions were removed at the abattoir and placed immediately in the fixative or in isopentane cooled in liquid nitrogen (for freeze-drying). In addition to the above, material was obtained from immature animals varying in age from 6 to 19 weeks and from embryos varying from 18·2 to 26 cm. C.R. length (approximately 80 days to term). Material from a 10-week-old cryptorchid piglet was also available.

Lipid

For demonstration of lipid frozen sections were cut from tissue fixed in Baker's calcium formol (Baker, 1946) or 2 % osmic acid. Paraffin sections of tissue fixed for 1 to 3 days in Elftman's dichromate sublimate mixture with the initial pH adjusted to 2·5 with HCl (Elftman, 1958) were also used and these were stained with Sudan black (sat. soln. in 70 % alcohol) with and without post-chroming. Frozen sections were stained with Sudan black and when this revealed sudanophilic material subsequent sections were similarly stained following 1–4 hours in a mixture of equal parts of methanol and chloroform at 37°, whilst other sections were stained with Sudan IV, Nile blue sulphate (Gurr, 1958) and by Baker's acid haematin method for phospholipid. Frozen sections from material fixed in osmic acid were examined unstained. The Schultz test for cholesterol esters was applied to further frozen sections.

Polysaccharides

For demonstration of complex polysaccharides, sections were fixed in Baker's calcium formol and in order to minimize the possibility of loss during fixation, other tissues were fixed in chilled absolute ethanol and chilled 95 % ethanol containing 10 ml. formol per 100 ml. (McManus & Mowry, 1958). In two cases the tissues were frozen-dried and subsequently fixed by exposure to osmic vapour.

Sections from all glands examined were routinely stained by the periodic acid-Schiff (PAS) technique using an aqueous solution of periodic (McManus, 1946) and also using solutions of periodic in alcohol and in glacial acetic acid (McManus & Mowry, 1958). Further sections were stained with PAS following incubation for 30 min. at 37° in saliva, diastase (0·1 % in 0·02 M phosphate buffer at pH 6·0 with NaCl added up to 0·8%) and hyaluronidase (Rhondase (Evans) 1 mg. per 100 ml. borate buffer at pH 6·8 for 12 hr.—Krammer and Windrum (1954)). Further sections were stained with Best's carmine, alcian blue (Mowry, 1956), toluidine blue and methylene blue (at pH values ranging from 3·5 to 7).

Phosphatases

For demonstration of enzymes tissues were fixed in a mixture of equal parts absolute ethanol and acetone (Danielli, 1953), and after paraffin embedding and sectioning the Gomori calcium phosphate method for 'alkaline' and the lead method for 'acid' phosphatase were employed (Gomori, 1953). For the former method a substrate of sodium glycerophosphate (a mixture of α and β isomers) was used, adjusted to pH 9, whilst for the lead method the pH of the glycerophosphate substrate was adjusted to 5. For demonstration of 5-nucleotidase a substrate mixture containing adenosine-5-phosphoric acid at pH 8·4 was used (Gomori, 1953). For demonstration of 'alkaline' phosphatase sections were incubated for 5 min. to 2 hr.; for 'acid' phosphatase for 1–24 hr.; and for 5-nucleotidase for 2–5 hr.

Sections from all glands examined were routinely stained by Gomori's trichrome method (1950), van Gieson's picrofuchsin and haematoxylin and eosin. The ninhydrin-Schiff (Yasuma and Ichikawa, 1953) and amido black 10B (Puchtler and Leblond, 1958) tests for protein, and the methyl green pyronin and gallocyanin-chromalum (Weber, 1958) tests for RNA were also employed. The latter reactions were controlled by incubating further sections for 30 min. at 37° in ribonuclease (0·001 % in 0·02 M phosphate buffer at pH 6·0) prior to staining.

RESULTS

Seminal vesicle

The seminal vesicle of the boar is a lobulated, branched, tubular gland. The larger tubules have a lumen measuring up to 2 mm. in diameter and are embedded in fibrous tissue containing some smooth muscle. In most tubules the wall presents short folds of variable width and length projecting into the lumen (Pl. 1, fig. 1), and although these may be absent from the larger tubules in a given specimen, they are always a distinguishing feature of this gland. Gland tubules are lined by a simple epithelium, consisting of columnar cells with only occasional rounded or angular basal cells. Nuclei are rounded and in the columnar cells are located in the basal cytoplasm. Bleb-like protrusions from the free surface are frequent and these often become detached and free in the lumen of the tubule (Pl. 1, fig. 2). There is a distinct basement membrane.

In sections stained with toluidine blue or methylene blue the cytoplasmic blebs and usually the immediately adjacent part of the cytoplasm, exhibit basophilia (Pl. 1, fig. 3). The basophilic areas do not exhibit metachromasia, however, nor do they stain with alcian blue or mucicarmine. They are, on the other hand, pyronin-positive and also stain with the chromalum-gallocyanin technique except after ribonuclease which effectively removes all pyronin-positive material.

Secreted material in the gland ducts also fails to stain with alcian blue or mucicarmine, but it is PAS positive even after incubation in diastase or saliva or in hyaluronidase. PAS positive material is also found within the epithelial cells but usually is not abundant (Pl. 1, fig. 4). Its distribution corresponds closely with that of the pyronin positive, basophilic material and it also gives the ninhydrin-Schiff and amido black reactions for protein. Similar reactions are given by the secreted material in the gland tubules. Neither the latter nor the PAS positive material within the cells react with Best's carmine. This applies regardless of the method of fixation employed.

Frozen sections of calcium-formol fixed material when stained with Sudan black usually exhibit a rather diffuse cytoplasmic reaction which may affect particularly the luminal cytoplasm (Pl. 1, fig. 5), but frequently affects all cytoplasmic areas equally. In most cases it seems more extensive in distribution than the PAS or protein reactions. The sudanophilic material is removed by exposure to methanol-chloroform for 4 hr. or more, suggesting a lipid, but it is at best coloured only slightly with Sudan IV and is negative with the acid haematin and Schultz tests. Nile blue sulphate gives a diffuse general staining of the cytoplasm of these cells which does not correspond accurately in every case with the sudanophilia, and which may be therefore non-specific.

Alkaline phosphatase is not present in the epithelial cells of the seminal vesicle of the boar, the reaction being confined to the capillary endothelium. Prolonged incubation (2 hr.) does not alter the position with regard to the epithelial cells but the connective tissue adjacent to the capillaries may then appear to be positive. Acid phosphatase can be demonstrated in the secretory cells, however, but is usually present only in small amount and then may appear to be exclusively nuclear, even after prolonged incubation (24 hr.). In some cases, however, a diffuse cytoplasmic reaction is noted with a particularly high concentration towards the luminal surface of the epithelial cells.

In foetal glands the tubules are narrow, without folds, and are widely separated from one another (Pl. 1, fig. 6). Each is immediately surrounded by a condensation of cellular connective tissue and beyond this by a loose mesenchyme. Muscular tissue is not apparent even in the full-term foetus. At 10 weeks of age the gland presents a rather similar appearance but each tube is then surrounded by a thin layer of smooth muscle. At 13 weeks the gland tubules are more numerous and have begun to dilate and the epithelium is taller than in the 26 cm. embryo (38–47 μ as compared with 21–31 μ). The epithelium at this stage, however, is still stratified as in the embryo (Pl. 2, fig. 7) and the few PAS positive granules present are readily removed by diastase and are presumably glycogen. At 19 weeks, however, the seminal vesicle is histologically and histochemically indistinguishable from the adult and, as in the adult, the tubules at this stage contain an abundant secretion.

The seminal vesicles from the 10-week-old cryptorchid pig, which had been markedly stunted through dietary deficiency at 2–3 weeks of age but which had responded well to treatment, were found to be identical in appearance with those of the healthy 10-week specimen.

Mast cells (cells containing basophilic granules which exhibit metachromasia with toluidine blue, methylene blue and gallocyanin and are strongly positive with alcian blue and are stained blue with Nile blue sulphate) are usually present in boar seminal vesicle in large numbers (Pl. 2, fig. 8). A particularly interesting feature of these cells is that they are frequently intra-epithelial (Pl. 2, fig. 9) and may be comparatively numerous in this situation. The intra-epithelial cells are invariably found in the basal part of the epithelium, usually in contact with the basement membrane, and are most common in glands in which mast cells are especially numerous. Mast cells located in the subepithelial connective tissue are also found frequently in contact with the basement membrane.

Prostate

The prostate gland of the boar is usually considered to consist of two parts, namely, compacta and disseminata, the former appearing as a number of rounded elevations on the dorsal aspect of the pelvic urethra at its anterior end where it is covered by the seminal vesicles, and the latter extending throughout the greater part of the length of the pelvic urethra. The two parts, however, are histologically similar and are not sharply separated from one another except at the level of the middle third of the compacta where, in some specimens, a sheet of dense fibrous tissue sometimes separates the two parts of the gland. This corresponds to the fibrous sheet which replaces the muscle on the dorsum of the urethra at levels caudal to the compacta. A distinguishing feature of the compacta, however, is the presence of trabeculae of smooth muscle. For the most part these are absent from the disseminata but surrounding the pelvic urethra, internal to the striated urethral muscle, there is a discontinuous layer of smooth muscle (Pl. 3, fig. 15) and although, in general, this lies external to the glandular tissue, strands of muscle sometimes extend between the more peripheral tubules. At the level of the compacta the visceral muscle, which elsewhere more or less completely surrounds the pelvic urethra, extends round its ventral and lateral aspects and then passes into the compacta where it breaks up to form the trabeculae found between the gland tubules. The striated urethral muscle is largely restricted to the ventral aspect of the urethra (Pl. 3, fig. 15) but frequently extends dorsally on the lateral aspect for a variable distance, especially at the anterior end, i.e. at the level of the compacta. It does not extend round the latter, however, this part of the gland being covered externally by a thin fibrous capsule. Immediately external to the urethral epithelium there is a layer of connective tissue containing the ducts of the prostate and numerous dilated venules forming a type of erectile tissue.

The secretory epithelium lining the prostatic tubules and alveoli is composed of a single layer of columnar or cuboidal cells with rounded nuclei located in the basal cytoplasm and, in general, is similar in both compacta and disseminata (Pl. 2, figs. 11, 12). In the latter, however, the epithelium sometimes decreases in height, this often being progressive from the anterior to the posterior end of the pelvic urethra and in occasional glands the epithelium lining the more posterior tubules is somewhat squamous in type. The tubules may also show a corresponding decrease in diameter and may be separated from one another by a rather greater accumulation of connective tissue than in the compacta (Pl. 2, fig. 10.) There is a distinct basement membrane. A similar though less pronounced decrease in cell height may be noted in the compacta, the tallest cells being found in the most anterior lobules. Here the ducts are usually comparatively wide and there is a greater abundance of inter- and intralobular connective tissue than at other levels of the compacta.

PAS positive granules are found in varying numbers in the luminal cytoplasm and a similar material may also be found in the lumen of some tubules. This material is unaffected by incubation in saliva, diastase or hyaluronidase, and is unstained by alcian blue and mucicarmine. The PAS positive substance is not basophilic and does not exhibit metachromasia with toluidine blue or methylene blue at pH values ranging from 3·5 to 5. Positive results are obtained with the ninhydrin-Schiff and amido black tests for protein and although these reactions, particularly the former, are weaker than the PAS reaction, they seem, in general, to be co-extensive with the latter. Pyronin positive material which is also positive with the gallocyanin-chromalum technique, varies in amount and location but is never particularly abundant and in some cells is completely absent.

Sudan black positive granules are found in variable quantity and are concentrated mainly in the basal cytoplasm (Pl. 2, fig. 12) although, when abundant, streams of granules can be seen on each side of the nucleus. There, however, they are usually discrete, whereas in the basal cytoplasm they may form a solid mass. Sudanophilic material is rarely found in the luminal cytoplasm and is absent from the secreted material in the lumen of the tubules. In general, the sudanophilic granules appear to be more abundant in the disseminata than in the compacta; the number in individual cells varies widely, however, and they may be absent, sometimes from all the cells of a tubule, and not infrequently from several adjacent tubules.

The sudanophilic granules are readily soluble in methanol chloroform and can be demonstrated in paraffin sections fixed in Elftman's dichromate sublimate mixture and in unstained frozen sections fixed in osmic acid. The granules can be stained with Sudan IV and by Baker's acid haematin technique for phospholipid and give a blue colour with Nile blue sulphate. Negative results are obtained with the Schultz test for cholesterol.

'Alkaline' phosphatase can be demonstrated only in capillary endothelium whilst 'acid' phosphatase reactions are generally slight and frequently confined to the nuclear membrane, although with prolonged incubation some cytoplasmic reaction may be noted and may extend throughout the cell. It is never intense, however, always being brown rather than black. The response to the technique for demonstrating 5-nucleotidase varies from specimen to specimen. Generally speaking, the enzyme is readily demonstrated in the disseminata but often appears to be absent from compacta or present only in isolated lobules. In other cases, however, distribution is uniform throughout disseminata and compacta. The reaction sometimes appears to be exclusively perinuclear in location but when more abundant it is located also in the luminal cytoplasm, especially near the free surface of the cell, and is then usually present also in considerable quantities in the alveolar and other gland ducts (Pl. 2, fig. 10).

Prostatic concretions are not a constant feature of boar prostate but do occur occasionally and then appear to be confined to the compacta.

In the late embryo PAS positive granules are present in abundance in the cytoplasm but are quite distinct from the PAS positive material found in the adult, being readily removed by diastase or saliva. Lipoid is also absent from the embryonic prostate.

Bulbourethral glands

The bulbourethral gland of the boar is more or less triangular in transverse section with the apex directed laterally and is usually traversed throughout the greater part of its length by a large central duct measuring 1 cm. or more in diameter. Variations in the duct system occur, however, and the large central duct may be absent. Muscular tissue (m. bulbourethralis) is restricted to the dorsum at most levels but may extend on to the ventral surface, especially towards the posterior end of the gland (Pl. 3, fig. 15). At no point is there any continuity between the m. urethralis and the m. bulbourethralis; the latter is of the striated variety and forms a thick layer. Elsewhere the gland is surrounded by a dense fibrous capsule continuous with that enclosing the muscle. Trabeculae of fibrous tissue extend into the gland, dividing it up into lobes and lobules.

The bulbourethral gland of the boar is a compound tubulo-alveolar gland and the interalveolar septa are unusually thin (Pl. 3, fig. 14), each consisting of a few collagen and reticular fibres. In consequence, the preparation of sections without disfiguring folds requires considerable care. The gland ducts are filled with a white, extremely viscid substance which sticks avidly to any surface with which it comes in contact. This substance is very hygroscopic, swelling enormously when immersed in aqueous solutions, e.g. aqueous fixatives, the secretion then bulging from the cut surface of the gland. On exposure to alcohol during dehydration it returns to normal proportions. It is unaffected by overnight incubation in a buffered hyalase solution at 37°.

The gland ducts and alveoli are lined by a single layer of large pryamidal cells with basally located nuclei which are often flattened (Pl. 3, fig. 14). Cell outlines are distinct and the free surface is usually domed. The cytoplasm is vacuolated and basophilic in haematoxylin and eosin preparations and is coloured green in trichrome preparations. Cells and secreted material contain a substance which gives positive reactions with PAS (even after incubation in saliva, diastase or hyalase) with alcian blue and mucicarmine, and is metachromatic with toluidine blue and methylene blue at pH 3·5. Sudanophilic granules are absent and there is no response with Best's carmine. The ninhydrin-Schiff and amido black reactions for protein are also negative.

A slight nuclear response to the 'acid' phosphatase technique is noted in some cases after 24 hr. incubation; there is no response to the 'alkaline' phosphatase technique or to the technique for 5-nucleotidase.

In the embryo some epithelial cells are loaded with PAS positive material; the remainder contain only small amounts of a PAS positive substance which is readily removed by treatment with saliva or diastase. The material in the former cells, however, is unaffected by saliva or diastase and is strongly positive with alcian blue (Pl. 3, fig. 13) and mucicarmine, and metachromatic with toluidine blue at pH 3·5

In piglets of 9 to 12 weeks of age the majority of gland cells give similar staining reactions to those noted in the adult and secreted material is present in the gland ducts. Alveoli, however, are much less numerous than in the adult and a correspondingly greater amount of interalveolar connective tissue is present. In the 19 week embryo, however, the gland is identical to that of the adult.

DISCUSSION

Seminal vesicle

The secretion of the seminal vesicle of the boar is a greyish, slightly turbid, watery fluid which flows freely from the cut surface of the fresh gland. Chemical examination of this secretion from glands subsequently used for histological and histochemical examination revealed the presence of protein and fructose, thereby confirming the findings of Mann (1946). Fructose is also a constituent of the secretion of the seminal vesicle of ram (Mann, 1946), but in this species glycogen, presumably the precursor of the seminal fructose, can be demonstrated in large quantities in both secretion and gland cells (Aitken, 1955) whereas appreciable quantities of glycogen cannot be detected in the seminal vesicle of the boar, no reaction being noted with Best's carmine, whilst all PAS positive material appears to resist the reaction of diastase. It is particularly interesting in this connexion that in the ram a high glycogen content of the secreting cells is a characteristic of this gland, even in the early foetus (17 cm.) and this feature persists throughout foetal and postnatal life, and is not simply a feature of the sexually active animal.

The possible loss of glycogen during the processing of the tissues has to be considered but attempts to reduce this, either by the use of frozen dried material, or by fixation in ice-cold absolute ethanol (McManus & Mowry, 1958) immediately after slaughter is without effect, nor are the results influenced by the use of alcoholic solutions of periodic acid, or solutions of periodic acid in glacial acetic acid (McManus & Mowry, 1958). Use of alcoholic fixatives does seem to result in more intense protein reactions but the difference is slight and the distribution of the reaction apparently is unaffected.

The PAS positive substance in the seminal vesicle of the boar thus appears to be a complex polysaccharide other than glycogen and the correspondence in localization between this substance and protein suggests a protein-polysaccharide complex. This is clearly not mucopolysaccharide, since it is not metachromatic with toluidine blue or methylene blue, and is negative with alcian blue and mucicarmine, and therefore may be either mucoprotein or glycolipid. The latter is suggested by the reaction with Sudan black but in many cases the sudanophilia is more extensive than the PAS reaction and it may be therefore that lipid and mucoprotein exist separately in the same cell. Wislocki (1949) found lipid in the supranuclear and infranuclear cytoplasm of the epithelial cells of the seminal vesicles of Virginia deer and his photographs illustrating this correspond closely to the appearance frequently noted in boar seminal vesicle stained with Sudan black.

The basophilia noted in the secretory cells in boar seminal vesicle is a further distinguishing feature when these are compared with the seminal vesicles of the ram in which the cells are neither basophilic nor pyronin positive (Aitken, 1955). Basophilia, however, is a prominent feature of some species, e.g. rat (Porter & Melampy, 1952; Melampy & Cavazos, 1953). Whether the mucoprotein contributes to the basophilia noted in the epithelial cells is unknown, but it is certain that this is due, in part at least, to RNA. The presence of the latter substance at the site of protein production is to be expected.

The function of the numerous mast cells in this gland in the boar has not been determined. Variation in number and localization in different specimens may reflect different functional states and may suggest that these cells are related to the production of the specific secretion. There is, however, no evidence other than this of the existence of such a connexion. Asboe-Hansen (1954) states that the mast cell population in a tissue is directly related to the hyaluronic acid content and considers that production of this polysaccharide is at least one of the functions of this cell. Production of hyaluronic acid does not seem to offer an adequate explanation of the numerous presence of mast cells in the boar seminal vesicle, since hyaluronic acid is not present in the secretion of the gland and the staining reactions of the connective tissue do not vary from animal to animal, although variations do occur in the mast cell population.

The presence of mast cells in the epithelium of glands in which they were especially numerous suggests migration from the connective tissue, particularly as in the latter many mast cells were found in contact with the basement membrane or in the near vicinity. Asboe-Hansen (1954) considered such migration possible but Johnson & McMinn (1957) considered that this did not explain the intra-epithelial cells which they found in the oesophagus of the cat. Wislocki (1949) found numerous mast cells in the stroma of the seminal vesicles of Virginia and Japanese deer but did not define their function.

Baker (1927) observed that in pigs of a similar age at slaughter (approximately 300 days) the seminal vesicles were larger in animals castrated at 200 days of age than in animals castrated at 100 days and that there was little difference in respect of these glands between the latter group and animals castrated at 50 days. He concluded that the influence of the testis is felt chiefly, or perhaps entirely, after the age of 100 days and that the effect is much greater between 200 and 300 days. Comparison of the histological features of this gland in the 10- and 13-week pig suggests, however, that the stimulus may begin to be effective somewhat earlier than 100 days and it is interesting that although the most rapid increase in size appears to occur between 200 and 300 days the gland is indistinguishable, histologically and histochemically, from the fully developed adult gland at 19 weeks. These results are in accord with the observation of Phillips & Andrews (1936) that marked development of testicular germinal epithelium begins in the pig at 84 days and that spermatozoa first appear at 147 days. Baker (1927) was of the opinion, however, that the response at 100 days was due to the gland developing the ability to respond to testicular androgens at this time and not to a sudden increase in the production of hormone. Thus the development of the seminal vesicle in the cryptorchid piglet to the same extent as in the normal animal may be due to the androgens having only a comparatively small influence on development of these glands up to approximately 13 weeks as suggested by Baker. Hanes & Hooker (1937), however, have shown that the testis in the cryptorchid pig contains 1 B.U. androgen per 87 g. testicular tissue which, although smaller than in the normal animal (1 B.U. per 39 g. testicular tissue) may be sufficient to permit continued development of the accessory genitals, especially in the earlier stages of comparatively slow growth. It is particularly interesting in this connexion that although the bulbourethral glands show a growth response similar to that of the seminal vesicles, they begin to produce a substance giving staining reactions identical with those characteristic of this gland in the adult at a much earlier age, in fact during intra-uterine development.

Prostate

The PAS positive substance in the secretory cells of boar prostate is not glycogen since it is not destroyed by incubation in a diastase solution or in saliva and is negative with Best's carmine, nor is it mucopolysaccharide since it does not exhibit metachromasia with toluidine blue or methylene blue, and is negative with alcian blue and mucicarmine. Other possibilities are mucoprotein or glycolipid. Lipid is clearly present in the gland cells but the sudanophilic material does not correspond to the PAS positive substance, the former being located in the basal cytoplasm and the latter in the luminal cytoplasm. Clearly therefore the PAS positive substance is not a glycolipid and is therefore probably a form of mucoprotein. The prostatic secretion in boar is thus chemically distinguished from that of the ram in which pronounced metachromasia suggests a sulphated mucopolysaccharide (Aitken, 1959). A further important distinguishing feature between boar and ram is that in the latter the characteristic mucopolysaccharide appears in the early foetus (approximately 20 cm.) its production apparently being largely independent of androgenic stimulation whereas even in the full-term boar foetus the only PAS positive material present is readily distinguished as glycogen. It is interesting also that at this stage the boar prostate contains considerable quantities of glycogen whereas the adjacent tissue of the seminal vesicle contains little or none. PAS positive material is also present in the luminal cytoplasm in dog prostate and Arcadi (1952) has expressed the opinion that this is a mucin, but unfortunately does not record the response to methylene blue, alcian blue or mucicarmine. Seaman (1954), on the other hand, was of the opinion that the secreted material in the dog was a lipoprotein.

The ready solubility of the sudanophilic material of the prostate in methanol-chloroform confirms its lipid nature whilst its stainability with Sudan IV and the blue colour given with Nile blue sulphate (due to combination of the oxazine base produced during preparation of the stain with acidic lipids) suggests that it is a fatty acid or phospholipid (Cain, 1950). This is confirmed to some extent by the response on fixing in osmic acid and although Gomori (1952) states that phospholipid may stain poorly or not at all with Sudan IV, phospholipid was finally confirmed by the reaction with Baker's acid haematin. The latter reaction was not confirmed by pyridine extraction since it was known that the material under consideration stained with Sudan black and was soluble in lipid solvents other than pyridine. Policardi & Noël (1920) found neutral fat and phospholipid in the basal cytoplasm of man and dog, and phospholipid in the luminal cytoplasm, whilst Huggins (1945) found phospholipid and some cholesterol in the prostatic epithelium of man. The negative results with the Schultz test show that cholesterol esters are absent from boar prostate.

Acid phosphatase of prostatic origin is found in relatively large quantity in some animal species and is regarded by Huggins (1945) as being a secondary sex character. Gutman & Gutman (1938) observed that small amounts only are present in infancy, increasing to reach high values in the adult. In boar, on the other hand, acid phosphatase occurs at most only in small amounts in the prostatic epithelium and not at all in secreted material in the gland ducts. This is true also of the ram (Aitken, 1959) and of the bull (Rollinson, 1954). Mann (1945) found 5-nucleotidase in boar semen and considered this to be of prostatic origin. This is confirmed by histochemical examination of the prostate.

McKenzie *et al.* (1938) expressed the view that the disseminata proper extends along the urethra for a short distance, and is then replaced by a different type of gland which these authors designated 'urethral' and which they considered to resemble the glands of Littré of man. They based this distinction on their finding that the gland tissue which they had designated 'disseminate' prostate was yellowish in colour, whereas the 'urethral' glands were not, and also on the finding that the 'disseminata' was compound tubulo-alveolar and the 'urethral' glands compound tubular. From a histochemical point of view, however, there is no essential difference in the glandular tissue at different urethral levels, nor is there any difference between compacta and disseminata other than the finding that 5-nucleotidase is more consistently present in the latter than in the former. The tubules of the disseminate prostate are generally narrower, however, than those of the compact prostate and are usually lined by a lower epithelium and these differences are most pronounced towards the lower end of the pelvic urethra. The change, however, is a gradual one and does not seem to justify the assumption of two distinct types of glandular tissue. Moreover, the glands of the pelvic urethra do not appear to correspond to the glands found occasionally in this situation in man (Johnson, 1920; Lichtenberg, 1906). The latter authors considered these glands to be structurally identical with Cowper's glands. Aitken (1959) found that in the ram the glands forming the so-called disseminate prostate were similar structurally and histochemically to the bulbourethral glands in this species. In the boar, however, the glandular tissue forming the disseminate prostate is readily distinguished histologically, histochemically and embryologically from the bulbourethral gland in this species.

Huggins (1945) states that prostatic concretions are found only in man and monkey. In fact, however, they are occasionally present in the boar but are then restricted in distribution to the compacta. There does therefore appear to be some slight justification for making a distinction between the compact and disseminate prostate although the two glands appear to be essentially similar.

Bulbourethral gland

The staining reactions of the bulbourethral gland of the boar indicate the secretion of a sulphated mucopolysaccharide. In this respect its secretion is chemically related to that of the bulbourethral gland of the ram (Aitken, 1959) but the peculiar physical character of the secretion in the boar, especially its stickiness and hygroscopic nature, suggest that the secretions of the two glands are not identical. In boar, as in ram (Aitken, 1959), secretion of mucin by the bulbourethral gland begins prior to birth, apparently being less dependent on androgenic stimulation than the prostate which, in this species, begins to produce its specific secretion some time after birth. Nevertheless, Baker (1927) did observe that the bulbourethral gland in the pig is markedly reduced in size following castration.

McKenzie *et al.* (1938) state that there is a thick muscular layer covering the ventral surface of the bulbourethral gland of the boar and securing it to the ventral surface of the pelvic urethra. This has not been confirmed and in fact in all the specimens examined the muscle associated with the bulbourethral gland was located on the dorsal surface of the latter except posteriorly where it extended on to the ventral surface. Even at this level, however, it does not secure the gland to the urethra. The striated muscle forming the m. bulbourethralis is sharply circumscribed and does not extend into the interlobular connective tissue as suggested by McKenzie *et al.* (1938).

SUMMARY

1. The seminal vesicles of the boar appear to secrete a mucoprotein. They may also produce a glycolipid but glycogen cannot be distinguished in these glands although they appear to produce the seminal fructose.

2. Mast cells are frequently present in very large numbers in the seminal vesicles and intra-epithelial mast cells are common.

3. The seminal vesicles undergo a transformation beginning at about the 90th day post-partum and proceeding rapidly to result in the gland being identical histologically and histochemically with the adult by the 133rd day of post-natal life.

4. The compact and disseminate parts of the prostate of the boar are similar to one another and appear to secrete a mucoprotein. In both phospholipid can be distinguished in the epithelial cells. Production of 5-nucleotidase appears to be more consistently a feature of the disseminata than the compacta but in some cases the latter is also involved in the production of this enzyme.

5. The bulbourethral glands produce a peculiar gelatinous mucopolysaccharide. Secretory activity appears to be less dependent on the activity of androgens than is the case in the other accessory genitals in this species.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Seminal vesicle. To show general appearance of tubules. × 100.

Fig. 2. Seminal vesicle. To show bleb-like protrusions of the free surface of the epithelial cells. × 400.

Fig. 3. Seminal vesicle stained with methyl green-pyronin. To illustrate pyronin positive basophilic areas of the cytoplasm. × 1100.

Fig. 4. Seminal vesicle stained by the PAS technique. × 400.

Fig. 5. Seminal vesicle stained Sudan black. × 1000.

Fig. 6. Seminal vesicle from 26 cm. pig embryo. × 100.

PLATE 2

Fig. 7. Seminal vesicle from 13-week-old pig. To show undifferentiated epithelium. × 400.

Fig. 8. Seminal vesicle. To show comparatively high concentration of mast cells. Stained toluidine blue. 5μ section. × 100.

Fig. 9. Seminal vesicle. To show intraepithelial mast cells. Stained toluidine blue. × 400.

Fig. 10. Disseminate prostate. To show distribution of 5-nucleotidase. × 100.

Fig. 11. Compact prostate. To show general appearance of glandular tissue. × 100.

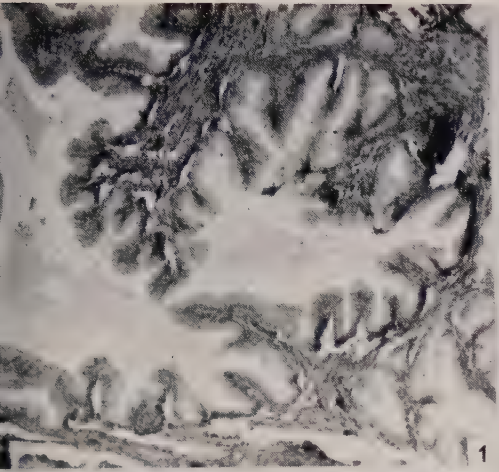
Fig. 12. Disseminate prostate. To show distribution of lipide. Stained Sudan black. × 500.

PLATE 3

Fig. 13. Bulbourethral gland from 26 cm. embryo. Stained alcian blue. × 100.

Fig. 14. Bulbourethral gland from normal adult to show appearance of secretory cells and interalveolar septa. × 400.

Fig. 15. T.S. urethra and bulbourethral gland from 26 cm. embryo. The urethral muscle covers only the ventral aspect of the urethra and the m. bulbourethralis, the dorso-lateral aspect of the bulbourethral gland. This section represents the posterior part of the pelvic urethra and includes the main excretory duct of the bulbourethral gland. × 15.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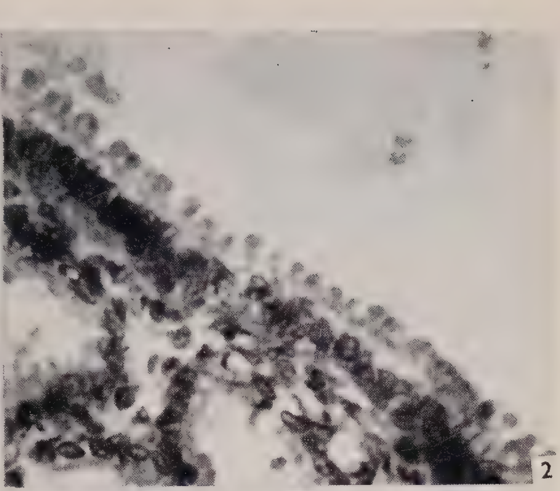
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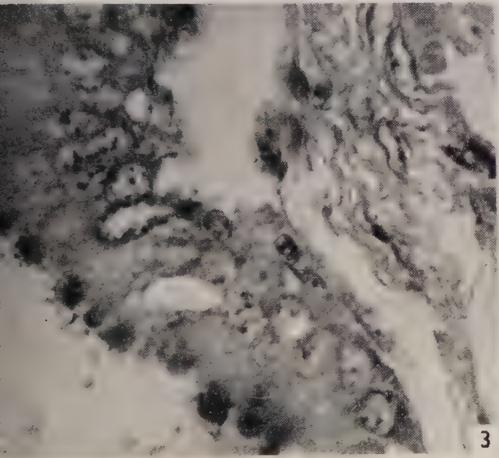
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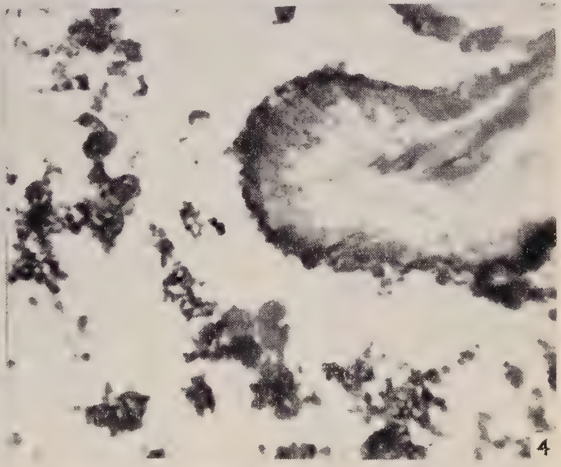
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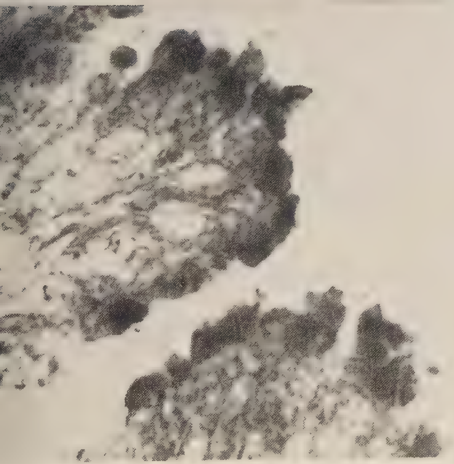
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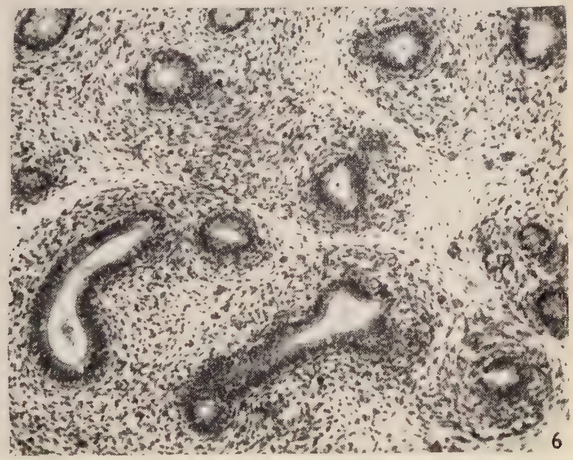
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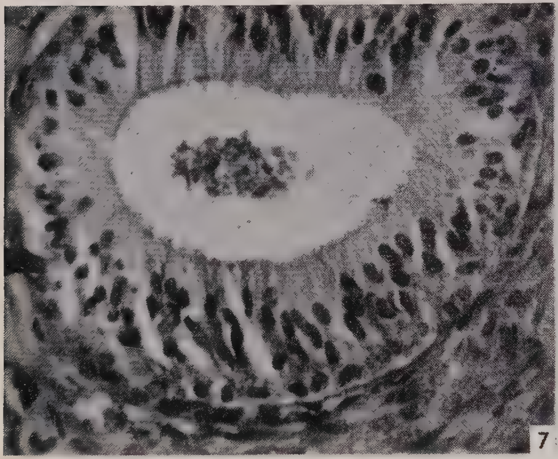
AITKEN—The accessory genital glands of the boar

(Facing p. 142)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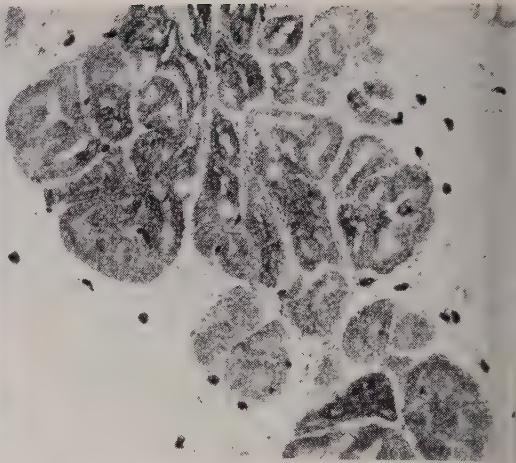

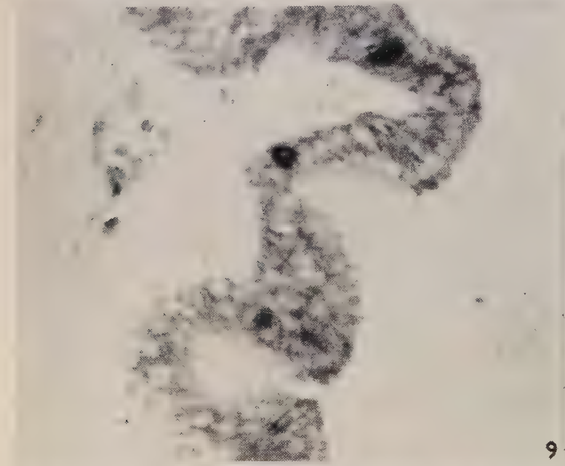
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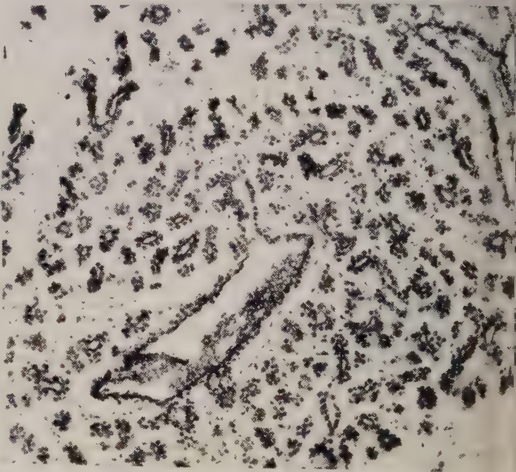

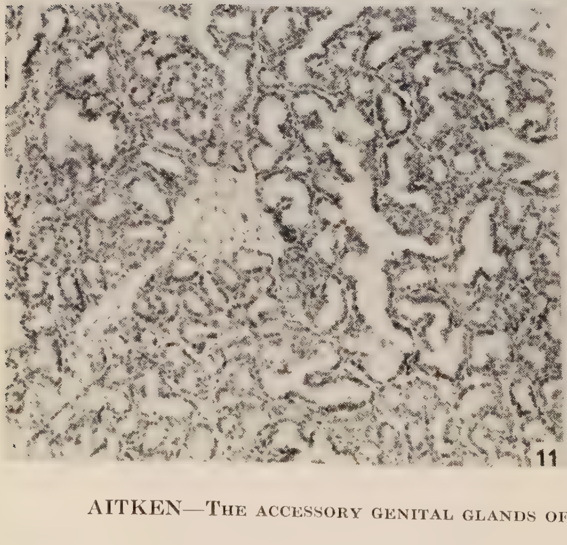
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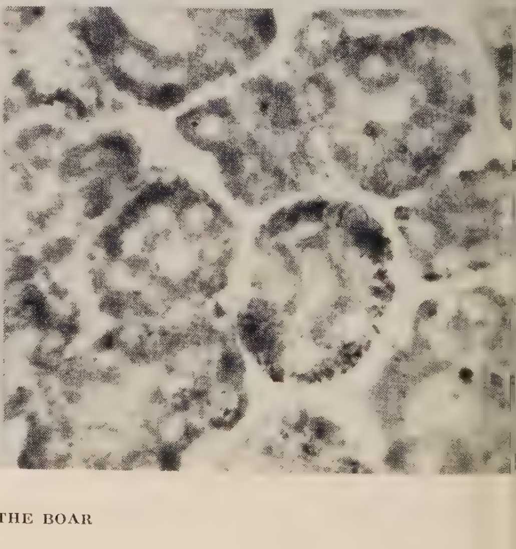
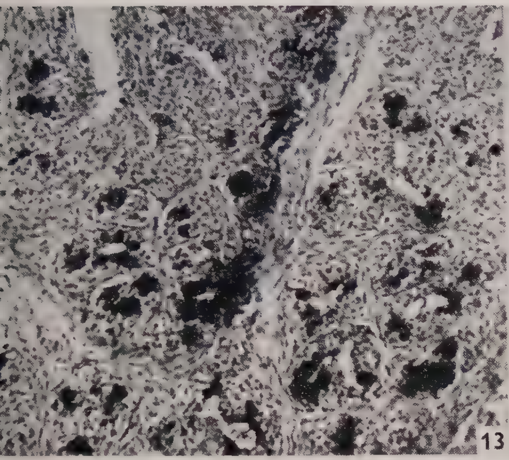


AITKEN—The accessory genital glands of the boar



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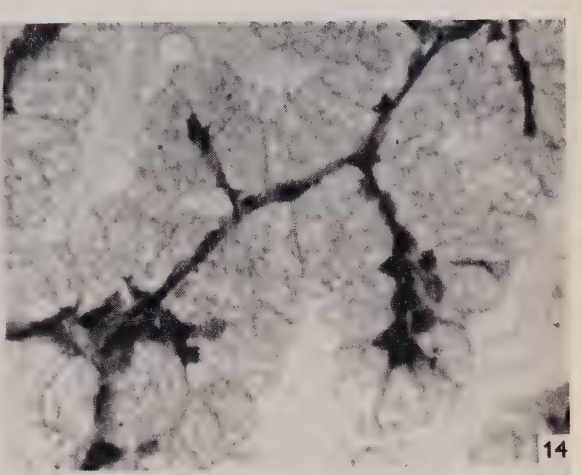

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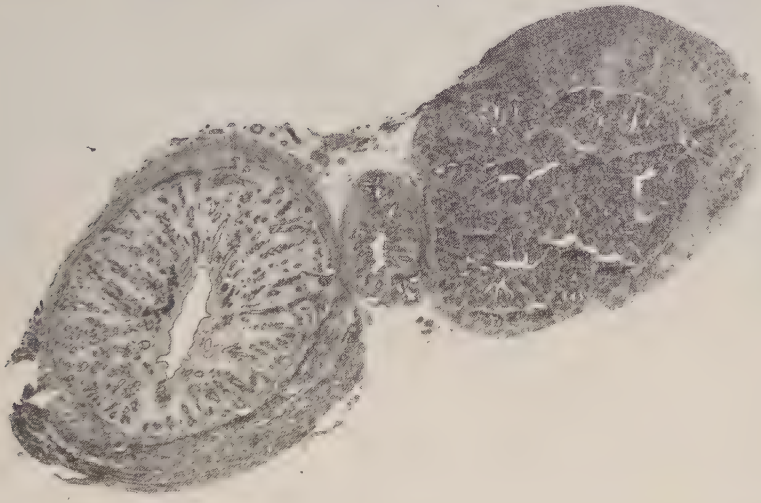

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AITKEN—THE ACCESSORY GENITAL GLANDS OF THE BOAR

REVIEWS

Subcellular Particles. Edited by T. Hayashi. (Pp. viii + 213; 87 figures; $6·00.) New York: Ronald Press. 1959.

This publication covers the fifth annual symposium of the American Society of General Physiologists which was held at Woods Hole in June 1958. According to the editorial foreword it was hoped that one of the effects of such a meeting would be to introduce to the variegated membership of the Society the most recent work in the study of subcellular particles. This is a most praiseworthy aim but one which may not have been fully realized since a fairly large part of the book can only be adequately appreciated by the specialist biochemist or enzymologist or biophysicist. Under the very wide title of the Symposium eleven papers were presented on various aspects of subcellular structure and function, and to do full justice to the distinguished contributors would be beyond the scope of a single reviewer in a few brief paragraphs.

Of particular interest is a fully reported discussion at the end of each paper, and since many of the articles contain reference to earlier and parallel investigations, the bibliographies are for the most part comprehensive. It is unfortunate, however, that throughout the book the reproduction of the photographs, and in particular the electron micrographs, is not first class, and this reduces its effectiveness as a publication.

Perhaps it is of value to give a few brief comments on some of the contributions which were understood by, and of interest to, an anatomist. The opening paper of Novikoff stresses the immense value of a multilateral approach to problems of cytological function and in it he gives some useful comments on the limitations and applications of phase contrast and electron microscopy. In discussing matrix-substrate and other systems reacting at membrane surfaces, Siegel suggests that their activities may ultimately be expressible as a function of the electrical properties and geometry of such surfaces, and believes that many biochemical problems may be reducible to questions of molecular morphology and interaction. Palade's study on the structuro-functional relationships of the pancreatic exocrine cell at stages during the secretory cycle has suffered a good deal because of the poor photographic reproduction: the article covers the formation and release of the zymogen granules and is a fine study in the combined use of several techniques in approaching such problems. A comprehensive and readable, if rather fanciful, treatment on the subject of mitochondrial structure and function is presented by Green. From the results of fractionation studies on heart muscle mitochondria he draws deductions on the inter-relationship of enzymic activity and mitochondrial membrane structure: for example, he believes there is a precise relationship between mitochondrial double membrane structure and the capacity for oxidative phosphorylation. The significance of the Golgi complex remains mysterious, but is further illuminated by Kuff and Dalton who have successfully fractionated out its constituent membranes *in vitro* and have shown that it is a region of no very distinctive enzymic activity. For a review of our present knowledge of lysosomes one could not wish for a better authority than De Duve. These 'bodies', characterized by their possession of a high hydrolase activity, have been reported in a number of different morphological guises and in several cell types: their relationship to cell autolysis and other pathological processes is discussed. Allfrey and Mirsky present a most significant study on the biochemical properties of the isolated nucleus: their methods include the treatment of nuclear fractions with deoxyribonuclease and the assessment of a consequent reduction in amino-acid uptake and so of protein synthesis by the nucleus. They have shown that DNA also mediates ATP synthesis and the uptake (and transfer to RNA) of purine and pyrimidine by the nucleus.

A further general, though not perhaps very useful, comment that might be made about this publication is to question whether any really helpful purpose has been served in presenting quite such diverse fare at one sitting and under one title. Possibly the individual contributors might have published their work to better effect in the well-recognized specialist journals.

J. D. LEVER

The Mast Cells. By JAMES RILEY, M.D., Ph.D., D.M.R.T., F.R.C.S.E. (Pp. 182+x; 65 figures; 25 tables; 30*s.*) Edinburgh: E. and S. Livingstone, Ltd. 1959.

Valuable dissertations are often not easily accessible or—what is more—their very existence is not known to the general reader, though usually the important results contained in them are available in published papers. This is also the case with the work of Dr Riley and his colleagues, particularly Dr West, on the 'Mast Cells', in which work overwhelming evidence has been presented in support of the hypothesis that these cells, and in particular their metachromatically staining granules, are the carriers and source of histamine.

Dr Riley has now published his Ph.D. Thesis on this subject, enlarged by a historical introduction, in the form of a monograph. As a consequence this book bears foremost the stamp of his personal experience and observations; and going through its pages we witness the gradual accumulation of experimental data consolidating the above hypothesis. The contemporary work of other investigators in this field is not omitted, but does not receive the detailed review and analysis which one would expect to find in a monograph. However, an extensive bibliography compensates for this, and everybody interested in mast cells and their functions will welcome the publication of this book. Its range is wide and covers mast cell behaviour, morphology and histamine content under normal and pathological conditions in a variety of species. It is free of dogmatism and of undue speculations. The author re-examines and re-interprets the heparin-'story' which, 20 years ago, seemed to have solved the riddle of the mast cell; he is aware of the presence in tissues of non-mast cell histamine; and, above all, he poses questions for future research.

The book is well produced and illustrated with excellent microphotographs; it opens with a foreword by Sir Henry Dale.

F. JACOBY

Zur Orthologie und Pathologie der Hoyer-Grosser'schen Organe. By J. SCHORN. (Pp. 88; 46 illustrations, Kartoniert DM. 27.50.) Stuttgart: George Thieme Verlag. 1959.

This volume is an addition to the now well-known series of *Zwanglose Abhandlungen aus dem Gebiet der normalen und pathologischen Anatomie*, edited by Prof. Bargmann and Prof. Doerr of Kiel. It consists of a brief summary of current knowledge on the arterio-venous anastomoses of the fingers and toes in Man and of a more detailed account of the author's own findings in fifty-three toes and eight fingers from forty-four individuals of whom six were normal and the remainder possessed established pathological conditions. The material had been sectioned in extensive series, and the findings are clearly recorded. Dr Schorn's data on the peripheral glomera in health and disease represent a real contribution to our knowledge of the structure and significance of arterio-venous anastomoses.

J. D. BOYD

TRANSNEURONAL CELL DEGENERATION IN THE LATERAL GENICULATE NUCLEUS OF THE MACAQUE MONKEY

By MARGARET R. MATTHEWS, W. M. COWAN and T. P. S. POWELL
Department of Human Anatomy, University of Oxford

INTRODUCTION

Recent quantitative studies of transneuronal degeneration in the dorsal nucleus of the lateral geniculate body (Cook, Walker & Barr, 1951) and in the superior cervical ganglion (Hamlyn, 1954) have served to establish that in the adult cat and rabbit the nerve cells here undergo a slow and comparatively mild process of degeneration following interruption of their afferent connexions, and that within the limits of the survival periods used no obvious cell loss occurs. In Primates, on the other hand, transneuronal degeneration in the lateral geniculate nucleus appears to be a more fulminating process, as it has been found to occur as early as 7 days after eye enucleation in the monkey (Glees & Le Gros Clark, 1941). Furthermore, in a human case at least 36 years after an eye removal Goldby (1957) has shown that the degree of cell shrinkage in the parvocellular laminae is considerably more marked than in the cat and rabbit after a survival period of 313 days, and that in these laminae about 50% of the cells had completely disappeared.

In view of the early onset of transneuronal degeneration in the monkey, experiments have been carried out to study the course of the degenerative process in this species. As this problem can only be investigated by quantitative methods, a study comparable to that of Cook *et al.* (1951) has been made in a series of monkeys during the first 4 months after unilateral eye enucleation. A point of particular interest in the lateral geniculate nucleus of the Primates is its organization into large- and small-celled laminae. The functional significance of this arrangement is not at all clear, but it appears that these two cell populations react differently both to deafferentation and to axonal section. In transneuronal changes affecting these cell groups, there is evidence that after long survival periods the large cells are less severely affected, and we have therefore sought to determine whether these differences are apparent from the beginning and continue through all stages of the degenerative process.

MATERIAL AND METHODS

Twelve mature monkeys (*Macaca mulatta*) were used in this study, though their precise ages were unknown. In each case one eye was enucleated, and in all but one animal (MG 1) an area of the visual cortex 1–2 cm.^{2} in extent was removed from one or both sides, either at the same time or at a second operation. The purpose of the cortical removals was to permit a later study of the interaction, if any, between retrograde and transneuronal degeneration in the same neurons. The animals were allowed to survive for periods varying from 4 days to 4 months (see Table 1). In addition, one normal monkey was used as a control. The brains were fixed in 70 % alcohol with 2 % acetic acid, and from each a block of the thalamus containing both lateral geniculate bodies was embedded in paraffin wax and sectioned coronally at 25 μ. Every 5th section was mounted and stained with thionin.

The nature and extent of the transneuronal atrophy were assessed qualitatively in each of the twelve experiments, and quantitative observations were made in the eight experiments indicated in Table 1 and in the control brain. As it was considered desirable to investigate the changes in all six laminae, all observations were made at the junction of the posterior and middle thirds of the geniculate body, where the six laminae are circumscribed and distinct. In view of the fact that each cortical lesion had resulted in a wedge of retrograde degeneration in the central zone of the ipsilateral nucleus, quantitative observations were confined to the lateral part of each lamina, well away from the zone of retrograde degeneration. All comparisons of normal with atrophied cells or laminae were made at corresponding sites and at the same antero-posterior level of the two geniculate nuclei in the same brain.

Table 1

Animal	Survival period (days)	Eye enucleated	Side of visual cortex lesion
*MI7	4	Right	Right
MI9	6	Left	Right
*MA4	7½	Right	Left
*MI8	8	Right	Left
MI10	10	Left	Right
MI12	12	Right	Right
MA3	14	Right	Bilateral
*MA2	16	Right	Bilateral
*MA5	33	Right	Bilateral
*MG1	62	Left	None
*MI13	91	Left	Right
*MA1	129	Right	Bilateral
*MG4	Normal control	—	—

* Indicates animals in which measurements of individual neurons were made.

For the measurement of cell shrinkage, camera lucida outlines of cell, nucleus and nucleolus for 100 neurons from each normal and atrophied lamina were drawn on mm. graph paper, the linear magnification being adjusted to exactly 1000 times. By counting the number of square mm. contained within each outline a measurement (in μ^2) was obtained of the nuclear, nucleolar and total cell areas. Sample fields were taken at random from near the middle of the lateral 'limb' of each lamina; in each field all neurons with a distinct nucleolus were measured, the presence of a nucleolus being the only criterion for their selection. The cell and nuclear outlines were always drawn in the plane of focus of the nucleolus.

In three experiments an attempt was made to detect loss of neurons by calculating their number in corresponding volumes of normal and atrophied laminae. In order to avoid the central zone of retrograde degeneration, the estimates of volume were confined to the middle three-quarters of the lateral 'limb' of each lamina. The areas of these segments were measured from projection drawings on mm. graph paper, at a magnification of fifty times, in six successive sections (125 μ apart) of the junctional region between the posterior and middle thirds of the geniculate nucleus, and the volumes were calculated from these data. Sample counts of cell density were made throughout these regions, using an oil immersion objective and a field of known diameter. Only neurons with a distinct nucleolus were counted, and the size of the sample was of the order of 2–5 % of the cell population in the volume measured.

The results of the qualitative observations will be described first, followed by the quantitative observations which, for the sake of clarity, will be presented either graphically or in tabular form. In the assessment of cell shrinkage, mean sizes of cell, nucleus and nucleolus for normal and atrophied neurons have been compared by the *t*-test, and percentage changes, with their 95 % confidence limits, have been calculated.

RESULTS

The observations are given under four headings: (1) the appearance of the normal neurons in thionin-stained paraffin sections; (2) quantitative observations made upon neurons in the normal laminae; (3) and (4) the changes qualitatively and quantitatively detected during transneuronal atrophy. The laminae are numbered 1–6 in the ventro-dorsal direction, from the hilum (Le Gros Clark, 1941), and for the sake of brevity, the terms 'crossed' and 'uncrossed' are used to describe those laminae which receive projections from the contralateral and the ipsilateral eye, respectively.

(1) *The normal lateral geniculate neurons*

The neurons in all laminae are multipolar and irregular in profile, but are commonly ovoid in general shape (Pl. 2, figs. 3, 5). There are slight differences in appearance between neurons of large-celled and small-celled laminae, but not between those of corresponding crossed and uncrossed laminae.

In the large-celled laminae (1 and 2) the neurons have relatively abundant cytoplasm which is evenly filled with dark-staining small Nissl granules less than 1 μ in diameter, extending into the bases of the larger dendrites. Occasional neurons have one or more clear areas in the cytoplasm, some of which may represent vacuoles, and others fixation artefacts. The nucleus is oval or rounded, often eccentrically placed. The nucleoplasm is much paler than the cytoplasm and contains a little faintly staining chromatin material. There is typically one nucleolus which stains darkly and is often eccentrically placed. Two nucleoli are rarely seen in the large neurons, but are not infrequent in the smallest neurons of the large-celled laminae.

The neurons of the small-celled laminae (3–6) have, relatively to their nuclei, somewhat less abundant cytoplasm. The cytoplasm is evenly and rather closely filled with small, dark-staining Nissl granules, or a reticulum of Nissl substance, with few large aggregates. As in the large-celled laminae, there are occasional clear spaces, most or all of which may be artefacts. The nucleus tends to be approximately central, but may be eccentric in the cells with more abundant cytoplasm. The nucleoplasm is usually paler than the cytoplasm, but contains many irregularly distributed chromatin granules, which vary greatly in their size and staining intensity. The nucleolus is often eccentrically placed and stains darkly; it is usually single, but in a small percentage of neurons there may be two nucleoli of typical appearance (the incidence of these 'double nucleoli' is commonly less than 3 %, but it has occasionally been found to be as high as 10 % in one or more of the small-celled laminae).

(2) *Quantitative observations in normal laminae*

The mean areas of normal cells, nuclei and nucleoli, with standard errors of the means, are given in Tables 2–9. Table 2 shows these values for the two geniculates of a normal animal. Tables 3–9* refer to both normal and atrophied laminae in seven experimental animals after unilateral eye enucleation, and are arranged in order of increasing survival periods, from 4 days to 4 months.

These results indicate that there is no atrophy in the unaffected laminae following enucleation of one eye. For any one lamina there is a considerable scatter of values from experiment to experiment; but it may be seen that while the mean values for atrophied laminae tend to decrease with increase of survival period, the mean values for unaffected laminae remain of the same order throughout, showing no consistent alteration with time.

The means of the values of cell area for each normal lamina in the eight animals were:

Lamina	Cell area (μ^2)	Lamina	Cell area (μ^2)
1	238·5	4	140·0
2	222·7	5	139·7
3	140·2	6	132·4

Limits of error; biological and technical variations. The scatter of mean observations for normal cells from experiment to experiment was such that, for most laminae, the largest mean cell area was approximately half as great again as the smallest mean cell area; in the case of lamina 2, however, the largest value was almost twice as great as the smallest. These differences between animals may reflect real differences, i.e. they may be biological, or they may have arisen during histological preparation; however, they would not directly influence the observations on transneuronal atrophy, since normal neurons for control measurements were taken from the same brain in each experiment. Variations due to observer errors were smaller, and were limited as far as possible in the following ways: (*a*) All the camera lucida outlines were traced by the same observer, using a standardized technique. (*b*) Although areas were measured by four observers, each set of atrophied neurons was measured by the one who measured the corresponding normal neurons. (*c*) Errors in tracing outlines were assessed at various times by making duplicate outlines of neurons; the resulting differences between pairs of measurements were proportionately greater the smaller the structure concerned, but were not more than about 5 % for the smaller cell areas (of less than 100 μ^2), or about 10 % for the smaller nuclear areas (below 40 μ^2). (*d*) The variation between observers in measuring a given group of cell outlines was assessed in a sample check of 50–100 cells each, and was found not to exceed 2–3 %. The measurement of nucleoli was somewhat crude, for although their outlines could be traced fairly accurately their areas with the method used could only conveniently be assessed to the nearest μ^2; but since 100 measurements were made in each group, and since the nucleolar area was variable, with the extremes of its range lying at about 1μ^2 and 7μ^2 and with demonstrable differences between large-celled and small-celled laminae, it was felt that the mean value for each group formed a useful approximation.

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* In these tables the second figure in each column is the standard error of the mean; this is 1/10 of the standard deviation, where the number in each sample is 100.

Distributions of cell areas. The scatter of cell areas is indicated by the standard errors of the means which are given for all groups of cells measured in Tables 2–9. Typical histograms of cell areas for 100 normal neurons from each lamina are shown as solid lines in Text-fig. 1. The distributions of areas in the large-celled laminae overlap considerably those in the small-celled laminae; indeed, in most animals

Table 2. *Normal control. Mean areas for* 100 *neurons from each lamina of the right and left lateral geniculate nuclei*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
amina	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
1	193·5	4·65	196·3	5·29	65·7	1·01	66·1	1·25	4·0	0·08	3·9	0·08
2	168·9	4·98	165·1	5·35	59·2	1·22	56·6	1·23	4·0	0·11	3·7	0·09
3	111·2	2·05	118·2	2·46	43·2	0·58	44·9	0·69	2·8	0·06	2·7	0·06
4	110·6	2·28	112·9	2·57	41·2	0·71	42·9	0·71	2·6	0·06	2·6	0·06
5	116·2	2·16	115·7	2·25	42·0	0·56	44·9	0·74	2·7	0·05	2·9	0·06
6	116·8	2·51	117·2	2·22	42·7	0·60	43·2	0·66	2·7	0·06	2·7	0·07

Table 3. *Eye removed* 4 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
amina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	245·8	6·71	223·1	5·20	82·0	1·58	78·3	1·50	3·7	0·08	3·3	0·07
2	231·2	5·20	228·4	5·28	79·1	1·35	81·0	1·20	3·8	0·08	3·5	0·08
3	138·6	2·93	129·2	2·63	55·9	0·76	57·1	0·79	2·7	0·06	2·5	0·06
4	136·9	2·98	123·2	2·63	57·0	0·99	53·5	0·76	2·7	0·06	2·4	0·06
5	142·9	2·78	124·7	2·16	53·3	0·63	54·9	0·76	2·4	0·06	2·3	0·05
6	119·8	2·30	99·7	1·74	50·7	0·75	45·0	0·76	2·4	0·05	2·2	0·06

Table 4. *Eye removed* 8 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
amina	Normal	S.E.	Atrophied	S.E.	Normal	S. E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	282·3	7·90	256·9	6·60	90·5	1·98	80·1	1·52	4·0	0·09	3·1	0·09
2	296·8	8·80	225·9	6·01	87·1	1·88	75·8	1·44	3·7	0·09	3·2	0·08
3	156·2	4·21	116·0	2·25	57·6	0·95	51·5	0·68	2·4	0·07	2·2	0·06
4	145·9	3·09	133·3	2·27	52·2	0·84	55·4	0·63	2·5	0·07	2·3	0·05
5	173·8	2·96	121·5	3·13	62·3	0·61	53·2	0·94	2·3	0·05	2·2	0·05
6	158·4	3·06	131·1	2·60	58·9	0·94	56·5	0·79	2·7	0·06	2·1	0·05

Table 5. *Eye removed* 16 *days previously*

(150 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
mina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	222·3	6·16	199·8	4·33	71·0	1·46	68·6	1·02	3·5	0·07	3·3	0·08
2	243·0	6·86	186·3	5·46	78·1	1·61	61·8	1·26	3·9	0·09	3·0	0·08
3	140·0	2·82	113·3	2·03	53·8	0·83	48·1	0·60	2·5	0·06	2·1	0·05
4	135·6	2·63	112·4	2·08	53·0	0·75	46·6	0·59	2·3	0·06	2·2	0·04
5	137·3	2·68	103·0	1·91	49·4	0·62	45·8	0·61	2·5	0·05	2·2	0·05
6	131·0	2·75	110·3	2·02	49·8	0·63	44·1	0·55	2·5	0·05	2·2	0·04

one or two cells were found in laminae 1 and 2 which were as small as any in laminae 3–6. On the other hand, it was very rare to find any cell in laminae 3–6 equal to the largest in laminae 1 and 2. Within each lamina, these histograms suggest a unimodal distribution and a single population of neurons. Polyak (1957) has described neurons of different types in single laminae in Golgi preparations, but all were found to undergo retrograde degeneration. In order further to test for the

Table 6. *Eye removed 33 days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	246·5	7·97	144·2	3·79	79·3	2·12	64·4	1·36	4·0	0·10	3·2	0·08
2	191·8	5·42	156·0	4·72	73·4	1·80	61·1	1·47	4·0	0·11	3·0	0·10
3	127·7	2·70	107·8	2·24	54·7	0·94	47·9	0·94	2·3	0·06	2·2	0·05
4	175·3	3·94	89·6	1·56	65·2	1·11	44·2	0·73	2·4	0·06	2·0	0·05
5	129·6	2·76	114·3	2·49	55·2	0·80	47·0	0·85	2·6	0·05	2·1	0·05
6	157·5	2·77	86·6	1·73	61·0	0·89	40·1	0·76	2·5	0·06	2·0	0·05

Table 7. *Eye removed 62 days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	206·7	6·12	146·7	4·51	74·2	1·61	56·1	1·42	4·4	0·12	3·0	0·09
2	186·7	7·06	140·3	3·90	66·6	1·91	52·9	1·09	3·7	0·10	3·1	0·08
3	140·8	3·19	90·2	1·63	49·5	0·78	38·1	0·52	3·0	0·07	2·2	0·06
4	133·7	2·94	83·6	1·85	47·1	0·79	33·5	0·57	2·8	0·07	2·0	0·06
5	121·5	2·95	81·5	1·53	45·6	0·87	31·4	0·53	2·6	0·06	1·9	0·05
6	114·2	2·11	74·2	1·52	44·6	0·58	31·9	0·58	2·8	0·06	2·1	0·05

Table 8. *Eye removed 91 days previously*

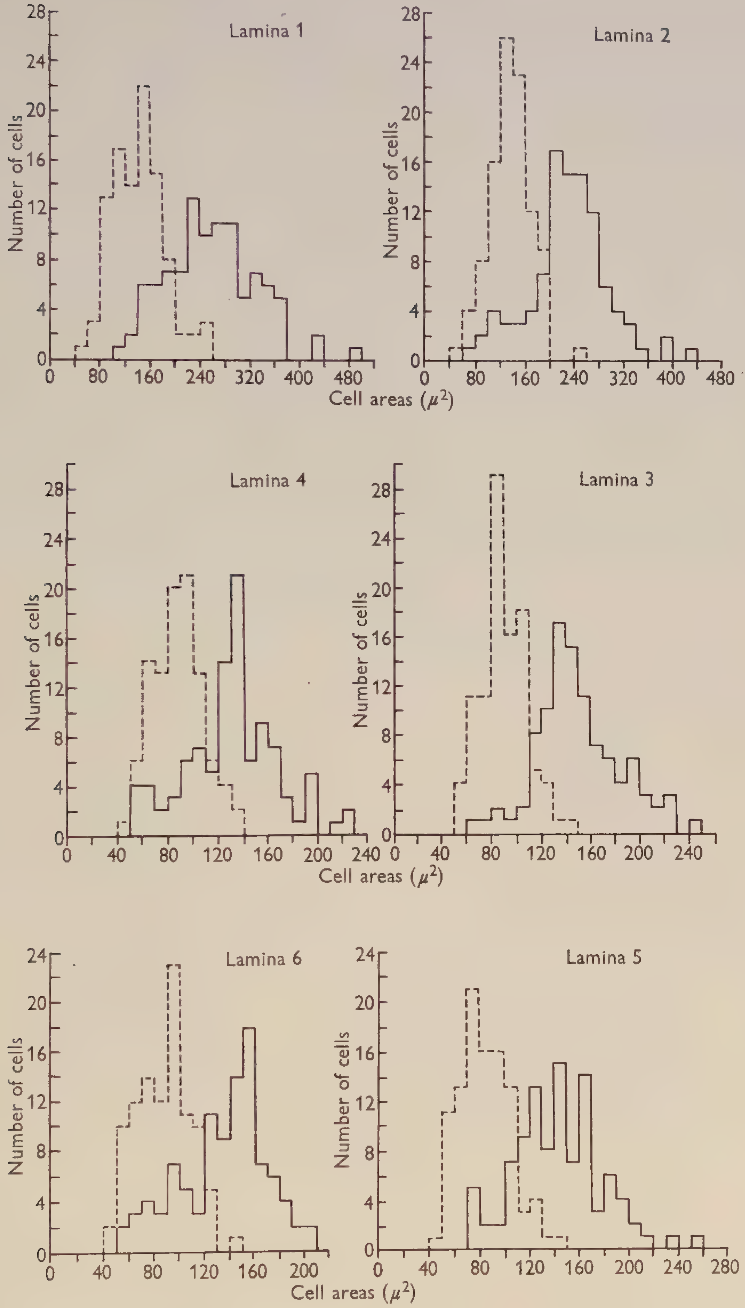
(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	251·1	7·07	98·8	2·94	73·6	1·39	39·0	1·04	4·8	0·11	2·4	0·08
2	234·4	6·15	142·9	3·15	71·0	1·46	48·5	0·95	4·3	0·10	3·1	0·08
3	153·5	3·05	88·6	1·57	53·1	1·20	37·3	0·52	2·8	0·12	2·1	0·06
4	151·2	4·10	82·2	1·76	49·5	0·88	33·2	0·50	2·9	0·10	1·9	0·04
5	154·2	3·80	91·2	1·33	51·9	1·34	35·7	0·60	2·7	0·06	2·1	0·05
6	125·8	2·83	72·9	1·44	48·1	0·79	34·8	0·55	3·1	0·06	2·2	0·04

Table. 9. *Eye removed 129 days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	258·5	7·22	140·7	4·07	70·6	1·23	53·2	0·96	3·1	0·08	2·2	0·06
2	230·3	6·43	136·4	3·28	76·4	1·44	49·6	0·85	3·5	0·09	2·2	0·07
3	149·9	3·39	89·6	1·83	57·1	0·82	36·6	0·51	2·4	0·05	1·6	0·06
4	129·6	3·66	87·3	1·92	44·4	0·87	37·2	0·55	2·3	0·08	1·8	0·05
5	142·3	3·43	84·1	2·00	53·2	0·74	31·9	0·53	2·4	0·06	1·8	0·06
6	135·3	3·45	87·0	2·12	46·6	0·99	36·0	0·63	2·2	0·07	1·9	0·05



Text-fig. 1. Histograms to show the distributions of cell areas for 100 normal and 100 atrophied neurons from each lateral geniculate lamina of a macaque, 129 days after enucleation of the right eye. Solid lines: normal neurons; broken lines: atrophied neurons. The group intervals are $20\mu^2$ for laminae 1 and 2, and $10\mu^2$ for laminae 3–6. The crossed laminae (1, 4 and 6) are on the left.

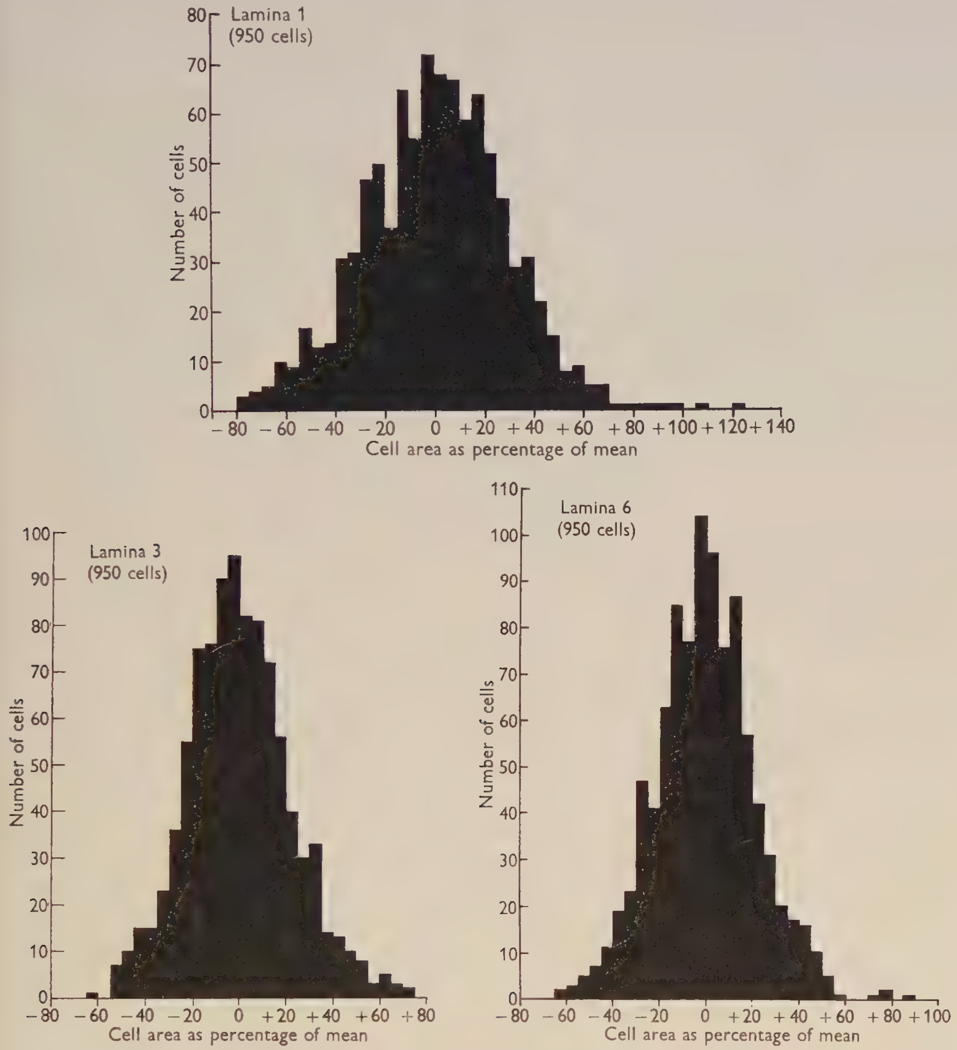
presence of distinct groups of neurons differing in size in normal laminae, various sets of comparable cell areas were pooled to increase the number of observations. Histograms were plotted for neurons of the same lamina showing similar mean cell areas in several different animals, and for the four small-celled laminae from single animals; these all showed unimodal distributions. As a more critical test, each group of cell areas for a given lamina was plotted with a grouping which represented percentage variation about its mean value, and the distributions so corrected for differences of the mean were added to give a histogram based on the measurement of 950 cell areas (the maximum number available for each lamina). This was done for laminae 1, 3 and 6, with a group interval in each case equal to 5 % of the mean, and the resulting histograms are shown in Text-fig. 2. It is clear that each of these histograms is effectively unimodal.

Neurons of different types might, however, differ in the extent to which they undergo transneuronal atrophy, or in the time-course of this atrophy, even if not differing in their original size. To test this possibility, the coefficients of variation of cell areas were analysed. Comparison of these coefficients of variation for normal and atrophied neurons at seven different survival times ranging from 4 to 129 days showed no consistent change; but for each lamina, at one or more survival times, there was a decrease in the coefficient significant by the *t*-test, and only once in one lamina (lamina 5, at 8 days) was there any significant increase. A decrease in the coefficient of variation of cell area might arise if the smaller neurons shrank disproportionately less, or more slowly, than the larger neurons, and would be transient if the difference were confined to the rate of shrinkage. But since shrinkage need not be directly proportional to the area of cross-section, changes in the coefficient of variation of cell area are not conclusive evidence for differences in behaviour of neurons within the same lamina during transneuronal atrophy. The present observations as a whole, therefore, are not inconsistent with the view that the neurons of any given lamina form a single population, within the region studied.

Differences of mean cell area between laminae. Apart from the obvious distinction between large-celled and small-celled laminae, there may be lesser differences within these groups. Chacko (1949), in a preliminary study based upon one human and one macaque brain, found gradients of cell size from lamina to lamina of the normal lateral geniculate. In the human brain, within the six-laminar central vision area of the geniculate, the neurons of lamina 2 were found to be somewhat larger than those of lamina 1, and there was a slight but fairly consistent gradient of decreasing size from lamina 3 to lamina 6. Similar relationships were found in the central vision area of the macaque lateral geniculate, and in the peripheral vision area of the human geniculate—where pairs of small-celled laminae fuse—laminae (3+5) had larger neurons than those of laminae (4+6).

In the present study neurons were measured in the six-laminar region of the geniculate, that is, in the 'central vision area'. Comparison of the mean cell areas for different normal laminae has shown that their relationships are rather variable. There is, however, a definite tendency for a decrease in cell area between laminae 3 and 6, although a continuous gradient is uncommon. In addition, if pairs of small-celled laminae are taken together, the cells of the uncrossed laminae (3+5) are often slightly larger than those of the crossed laminae (4+6).

These conclusions are based upon observations in eight animals. It may be objected, however, that seven of these had unilateral eye enucleations. In these seven animals the normal crossed and uncrossed laminae were in opposite geniculates, so that any differences between them may have been due to differences in cell size between the two geniculates. In the normal control animal, however, no significant differences in area were found between cells of the same lamina on the two sides (the greatest difference was 6 % in lamina 3, which was significant only at the 1 in 20 level). It is therefore unlikely that there are regularly significant differences in size between normal cells in the two geniculates; and, indeed, it is on the assumption that there



Text-fig. 2. Histograms to show the distributions of normal cell areas, plotted as percentage deviations from the mean, for three lateral geniculate laminae. The grouping interval is 5 % of the mean. The derivation of the histograms is described in the text.

are not such differences that any quantitative study of transneuronal atrophy is based.

One animal (Table 7) showed a continuous gradient of decreasing cell area from lamina 3 to lamina 6, similar to that found by Chacko, but unlike her finding the neurons of lamina 2 were smaller than those of lamina 1. For the large-celled laminae this was the commonest relation, and was found in six of the eight animals, including the normal control; in these six animals the cell areas for lamina 2 were, on average, 12 % smaller than those for lamina 1. Among the small-celled laminae there was most commonly little difference of mean cell area, but there was a tendency for lamina 6 to show the smallest values. Thus, in four animals, including the normal control, the mean cell areas of laminae 3–5 were approximately equal to each other, in two cases exceeding, but in two cases also equalling that of lamina 6. But for the small-celled laminae a comparison of laminae 3 and 5, or 4 and 6, is possibly more interesting. The mean cell areas of laminae 3 and 5 were approximately equal in five of the eight animals, whereas the cell area for lamina 4 more often exceeded than equalled that for lamina 6 (greater in four, equal in three animals). Only once, and in the same animal (Table 4), were the neurons of lamina 5 and lamina 6 greater than those of laminae 3 and 4, respectively. Crossed and uncrossed laminae were compared; for the small-celled laminae it was found that in five animals the mean of the cell areas for laminae 3 and 5, taken together, exceeded that for laminae 4 and 6 together, but this did not apply to the large-celled laminae, for only once, and in a different animal, was the value for lamina 2 greater than that for lamina 1.

Incidence of double nucleoli. Neurons having two nucleoli ('double nucleoli') were present in small numbers in most normal laminae. In two of the thirteen animals studied, double nucleoli were seen in all laminae. They were commoner in the small-celled than in the large-celled laminae, for they were found in all the small-celled laminae of eleven animals, but were seen in both large-celled laminae only in four animals. They appeared, moreover, to be rather commoner in lamina 1 than in lamina 2. As has already been noted, double nucleoli in the large-celled laminae tended to occur in the smaller rather than in the larger neurons.

The percentage incidence of double nucleoli on the whole was low, the highest values being 8–10 %, seen in one or more small-celled laminae in each of three animals. Approximate percentage incidences were obtained in eight animals for all laminae by observing the number of cells with double nucleoli in the samples drawn for measurement of area; in one of these animals larger samples of about 1000 were taken in the small-celled laminae. The means of the percentage incidences for each lamina, in the eight animals, were:

Lamina	Percentage of neurons with double nucleoli	Lamina	Percentage of neurons with double nucleoli
1	1·1	4	2·0
2	0·7	5	2·2
3	2·4	6	1·1

The mean incidence of double nucleoli in the large-celled laminae, taken together (0·9 %), was approximately half as great as that for the small-celled laminae (1·9 %).

'*Nuclear-cytoplasmic ratios*'. For each normal lamina, the mean nuclear area was subtracted from the mean cell area to give a figure for the 'mean area of cytoplasm'.

This figure was then divided by the mean nuclear area to yield an approximate 'nuclear-cytoplasmic ratio'. The 'nuclear-cytoplasmic ratios' for the large-celled laminae were almost invariably lower than those for the small-celled laminae, and this confirmed the impression previously noted of a relative abundance of cytoplasm in the large cells; the mean 'nuclear-cytoplasmic ratio' in eight animals for laminae 1 and 2 together was 1:2·07, and for laminae 3–6 together, 1:1·70. No other consistent relationship was noted between these ratios for pairs or groups of laminae.

(3) *Qualitative assessment of the atrophy following eye enucleation*

The changes in appearance of neurons during transneuronal atrophy are conspicuous at an early stage, because they affect whole laminae and also because the unaffected laminae are immediately adjacent for comparison. The adjacent normal laminae provided a convenient standard of reference in describing the changes of atrophy. The changes detected in the atrophied laminae, by comparison with adjacent normal laminae, could be confirmed by reference to the normal laminae at the appropriate level in the opposite geniculate. A description was recorded of the cells in each lamina, normal and atrophied, and these descriptions were later compared.

As early as 4 days after eye enucleation, which was the shortest survival period, a slight change in staining intensity of the atrophic laminae was apparent on low-power inspection. This did not appear to be equal on the two sides; whereas the crossed atrophic laminae (1, 4 and 6) were definitely paler than the adjacent normal laminae (Pl. 2, fig. 7), the uncrossed atrophic laminae (2, 3 and 5) were only just perceptibly paler than the adjacent normal crossed laminae. Measurements of cell areas at this stage showed that the cells of the crossed atrophic laminae were all significantly shrunken, those of lamina 6 showing most change (see Table 10), while of the uncrossed atrophic laminae only the cells of lamina 5 showed any significant shrinkage. At 6 days, all atrophic laminae were definitely paler than the adjacent normal laminae, but the uncrossed laminae now showed a rather more profound decrease in staining intensity than the crossed laminae, and the neurons of all three uncrossed laminae appeared shrunken (Pl. 1, fig. 1). Measurement in two laminae (3 and 4) at 7½ days, and in all laminae at 8 days, showed a considerable decrease in mean cell area in the uncrossed laminae, which exceeded the shrinkage in the crossed laminae (Pl. 2, figs. 8, 9). At the same stage, an increase in density of glial cells was noted in the uncrossed laminae: this will be termed 'apparent gliosis', since it might be caused by shrinkage of neurons and loss of neuropil, as well as by a true increase of glial cells. First appearing in the uncrossed laminae at 7½ days and seen more definitely at 8 days, this apparent gliosis was well marked at 10 days, but at 12 and 14 days it was less, and at 16 days it was no longer certainly present (although it was seen in these laminae after longer survival periods). Both qualitative and quantitative observations showed that between approximately the sixth and the tenth day after eye enucleation the cells of the uncrossed laminae underwent a marked and rapid shrinkage, while from then until 16 days there was no evidence of further shrinkage. The early period of apparent gliosis observed in these laminae thus coincided closely in time with the early rapid shrinkage of their cells.

Meanwhile the cells of the atrophic crossed laminae, which underwent an even earlier and quite marked shrinkage during the first 4 days after eye enucleation, showed no further decrease in size between 4 and 8 days, nor, with the exception of a small decrease in lamina 4, between 8 and 16 days. Apparent gliosis in the crossed laminae began slightly later than in the uncrossed laminae, and was somewhat less well marked at 10 days; unlike that in the uncrossed laminae, however, it persisted at the same intensity at 12, 14 and 16 days. This less intense and more prolonged period of gliosis thus coincided with cell shrinkage only in lamina 4.

Between 16 and 33 days, the cells of the crossed laminae underwent the greatest degree of shrinkage common to them all, and showed little consistent change thereafter; whereas from 16 days onward the uncrossed laminae showed no major consistent change, but rather an overall slow progression of shrinkage until 3 months. Pl. 1, fig. 2, shows the appearance of the left lateral geniculate nucleus 3 months after enucleation of the left eye. At 33 days, and at 2, 3 and 4 months, there was apparent gliosis of moderate and roughly equivalent degree in all six atrophied laminae.

The appearance of atrophied geniculate neurons 4 months after unilateral eye enucleation, with normal neurons from the opposite side for comparison, is illustrated in Pl. 2, figs. 3–6.

Cytoplasmic changes. The decrease in overall staining intensity of atrophic laminae, which was among the earliest changes observed, was due to a decrease in staining intensity of the cytoplasm of the cells, and this in turn appeared to be due to changes in the Nissl granules. The Nissl granules, or reticulum of Nissl substance, became progressively paler and less conspicuous, and no longer formed dark aggregates in the large cells; there was no obvious decrease in the size of the granules, however, nor any change in their density or their distribution within the cytoplasm. But the decrease in staining intensity was great enough after 1 or more months to give an almost homogeneous, ground-glass appearance to thick cell sections, and the granules were then only well seen in thin sections of cells. The next change to become qualitatively apparent was a shrinkage of the neuron, with an attenuation of the dendrites. This shrinkage was measured in eight animals, and will be presented with the quantitative observations. No increase in the small number of apparent vacuoles was seen in atrophic cells.

Nuclear changes. There was no decrease in staining intensity of the nucleus of the atrophic neurons. The nuclear membrane became more conspicuous at an early stage, remaining so thereafter, but this appeared to be largely illusory, due to the increasing pallor of the cytoplasm, especially in the large-celled laminae where the normal cytoplasm was so dark as often to obscure the nuclear membrane completely. In many neurons of the small-celled laminae, after 1 or more months of atrophy, there appeared an irregular, slight thickening of the nuclear membrane, which made it even more conspicuous. As the neuron became smaller, the nuclear area decreased less than the cytoplasmic area; and it became less common to find an eccentrically placed nucleus, particularly in the small-celled laminae (in which this was rare after 6–8 days). In the large-celled laminae eccentric nuclei were definitely uncommon from 33 days onward.

Nucleolar changes. Apart from a decrease in area, which followed roughly that of the nucleus, the nucleolus showed one early and possibly one late change in the atrophied neurons. First, in the small-celled laminae, from about 8 days onward the rounded clear areas which were present in the normal nucleoli of most of these animals became indistinct, and by 1 month they had virtually disappeared. This process also occurred to a lesser extent in the larger nucleoli of laminae 1 and 2. Secondly, in the animal with the longest survival period (4 months) there was a considerable increase in the incidence of double nucleoli in all the atrophied laminae, the mean percentage of neurons with double nucleoli in the normal laminae being 1·2 %, and in the atrophied laminae 4·4 %. This observation is based on over 8000 cells in this animal; in other animals not more than between 1000 and 2000 cells have been sampled and these were probably too few to show small changes. In one other animal, at 33 days, double nucleoli were not found in the normal small-celled laminae but were seen in three of the atrophied small-celled laminae; the incidence in these laminae, however, was only of the order of 1 %.

Nucleolar satellite. In the four animals in which typical nucleolar satellites were observed, no obvious change was found in their appearance or position in the atrophied neurons, but no quantitative study was made. This is in accordance with the findings of Cook *et al.* (1951) in the cat.

(4) *Quantitative observations in atrophied laminae*

Shrinkage of neurons. The mean areas of atrophied cells, nuclei and nucleoli, with standard errors of the means, are given in Tables 3–9, which are arranged in order of increasing survival periods, from 4 days to 4 months. In Text-fig. 1 are shown the histograms of cell areas for 100 normal and 100 atrophied neurons from each lamina after 4 months' survival. Although the histograms for the atrophied neurons appear more compact than those for the normal neurons when plotted on the same scale, the coefficient of variation of the populations is in fact little altered, and only for lamina 4 is it significantly decreased. The percentage changes in area of cell, nucleus and nucleolus at each survival period are given in Tables 10–12, together with the results of the t-test for the significance of each comparison. Each percentage change is calculated with reference to its appropriate normal control. These Tables, and the graphs constructed from them, permit some assessment of the rate of transneuronal atrophy, though allowance must be made for the possibility of 'biological variation', since with a single exception only one animal was used for quantitative study at each survival time.

The time-course of the shrinkage, and the similarities and differences of behaviour within and between groups of laminae, are illustrated graphically in the succeeding figures, in which mean areas for atrophied neurons are plotted as percentages of the mean normal control areas. The initial 'control' observation shows the values obtained by comparing the two geniculate nuclei of a normal animal; this has been done using the laminae corresponding to the left eye as the standard of reference, since the enucleation was on the right side for all except two of the experimental animals (see Table 1). Text-fig. 3 shows cell, nuclear and nucleolar areas plotted together against survival period in days for each lamina of the crossed and uncrossed groups. The cell area, which is some index of the cytoplasmic volume, has almost always shrunken proportionately more than the nuclear area, and the nucleolar area, though crudely measured, has followed the nuclear more closely than the total cell area. (The shrinkage of the nucleolus may have been somewhat under-estimated, for in the atrophied neurons chromatin particles were often apposed to the nucleolus, obscuring its outline.) The difference between the shrinkage of nucleus and cell tended to be greater following periods of rapid cell shrinkage, as, for example, at 33 days for the crossed laminae and 8 days for the uncrossed laminae; this suggests that the nuclear atrophy is slower than that of the cytoplasm though, in the uncrossed laminae at any rate, it may not ultimately be any less. It is also evident that the crossed laminae resemble each other more closely than they resemble the uncrossed laminae, and vice versa. Within each group, however, the two small-celled laminae (4 and 6, or 3 and 5) resemble each other rather more closely than they resemble the large-celled lamina (1 or 2). This difference might reflect a difference of function between large and small cells, but it might possibly have arisen as an

Tables 10–12. *Percentage changes in area of cell body, nucleus and nucleolus in lateral geniculate neurons at different periods after unilateral eye enucleation*

Asterisks indicate level of significance by the *t*-test. * significant at 5 % level; ** significant at 1 % level; *** significant at 0·1 % level.

Table 10

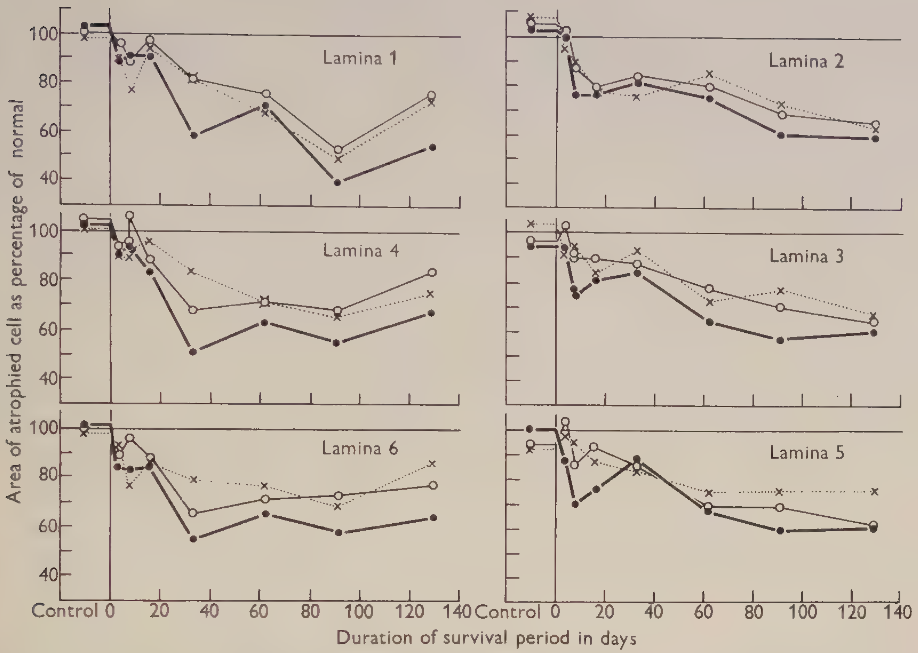
Survival time (days)	Percentage change of cell area from normal					
	Lamina 1	Lamina 2	Lamina 3	Lamina 4	Lamina 5	Lamina 6
Control	+1·5	+2·3	−5·9*	+2·1	+0·4	+0·3
4	−9·3**	−1·2	−6·8*	−10·0***	−12·7***	−16·8***
8	−9·0*	−23·9***	−25·7***	−8·6***	−30·1***	−17·2***
16	−10·1*	−23·3***	−19·1***	−17·1***	−25·0***	−15·8***
33	−41·5***	−18·7***	−15·6***	−48·9***	−11·8***	−45·0***
62	−29·0***	−24·9***	−36·0***	−37·5**	−32·9***	−35·0***
91	−60·7***	−39·1***	−42·3***	−45·6***	−40·9***	−42·1***
129	−45·6***	−40·8***	−40·2***	−32·6***	−40·9***	−35·7***

Table 11

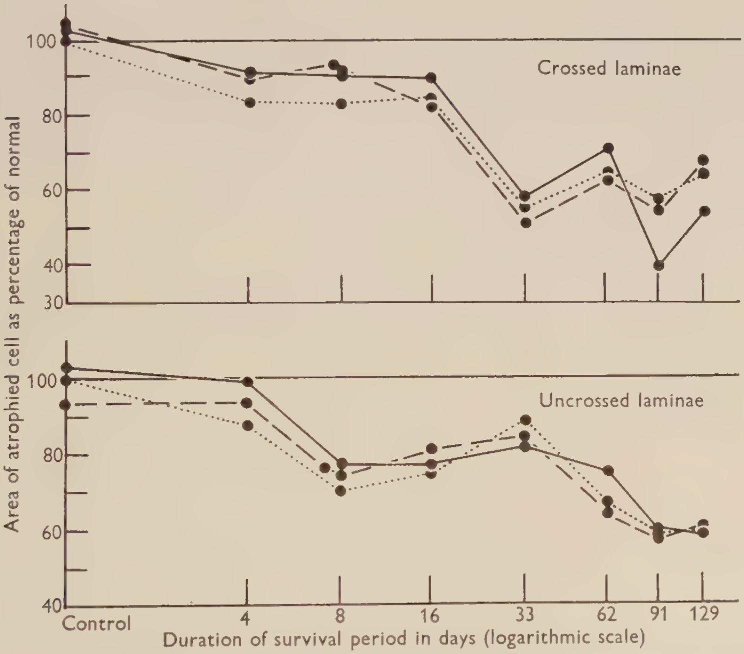
Survival time (days)	Percentage change of nuclear area from normal					
	Lamina 1	Lamina 2	Lamina 3	Lamina 4	Lamina 5	Lamina 6
Control	+0·5	+4·6	−3·7	+4·2	−6·5**	+1·0
4	−4·5	+2·4	+2·2	−6·1**	+3·0	−11·2***
8	−11·6***	−13·0***	−10·6***	+6·0**	−14·5***	−4·0
16	−3·4	−20·9***	−10·7***	−12·2***	−7·4***	−11·6***
33	−18·8***	−16·8***	−12·5***	−32·3***	−15·0***	−34·3***
62	−24·5***	−20·6***	−22·9***	−28·8***	−31·1***	−28·5***
91	−47·1***	−31·6***	−29·8***	−32·8***	−31·2***	−27·6***
129	−24·6***	−35·1***	−35·9***	−16·3***	−40·0***	−22·8***

Table 12

Survival time (days)	Percentage change of nucleolar area from normal					
	Lamina 1	Lamina 2	Lamina 3	Lamina 4	Lamina 5	Lamina 6
Control	−2·1	+6·7	+3·3	+0·8	−7·3*	−1·1
4	−10·5***	−5·6	−9·3**	−10·2**	−3·4	−6·8*
8	−22·9***	−11·5***	−6·4	−8·1*	−5·2	−23·0***
16	6·0*	−22·3***	−15·9***	−4·3	−13·4***	−13·7***
33	−18·4***	−25·0***	−7·4*	−16·6***	−16·9***	−20·5***
62	−32·0***	−15·6***	−27·2***	−28·5***	−25·7***	−23·6***
91	−50·5***	−27·7***	−23·1***	−34·6***	−25·0***	−30·9***
129	−27·5***	−36·3***	−33·1***	−24·9***	−24·4***	−13·9***

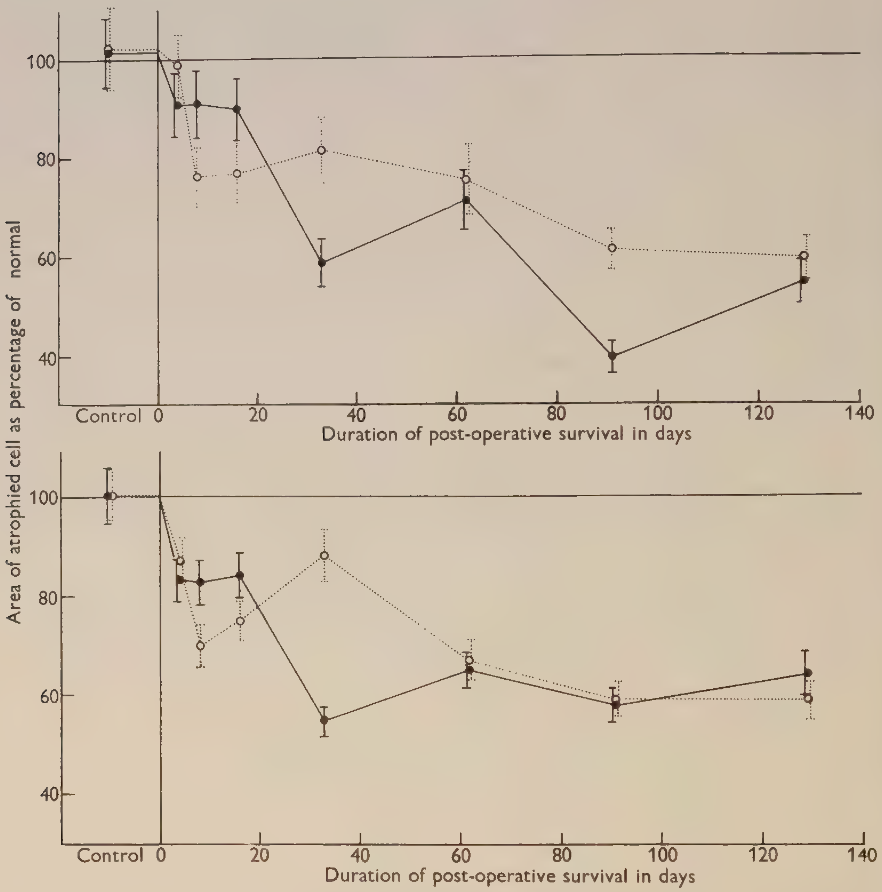


Text-fig. 3. Graphs to show the time-course of the changes in mean cell, nuclear and nucleolar areas for each lamina following eye enucleation. Each mean value for the atrophied neurons is plotted as a percentage of the mean normal value for that animal. The crossed laminae are on the left. ● = cell area, ○ = nuclear area, × = nucleolar area.



Text-fig. 4. Graphs to show the differences in the time-course of changes in cell area between the crossed and the uncrossed laminae, and the similarities within these groups. Upper graph: solid line, lamina 1; broken line, lamina 4; dotted line, lamina 6. Lower graph solid line, lamina 2; broken line, lamina 3; dotted line, lamina 5.

error inherent in sampling, owing to the greater variance of the population of neurons in the large-celled laminae which is apparent from a comparison of the histograms in Text-fig. 2. When the laminar groups as a whole are compared, crossed with uncrossed, their behaviour is seen to differ appreciably during the first month after eye enucleation, but only slightly from 2 months (62 days) onward, especially if lamina 1 is excluded. The differences within the first month are illustrated by a semi-logarithmic plot in Text-fig. 4, in which a logarithmic scale is used for the survival period, and the mean cell areas for the three laminae of each group are plotted linearly as percentages of normal. This serves to expand the early part of the time scale, which appears to be of most interest. Text-fig. 4 shows that, at 4 days, cell areas in all the crossed laminae are already decreased by 9–17 %; the shrinkages are statistically significant and remain substantially unchanged until some time after 16 days. Of the uncrossed laminae, only lamina 5 has a significant decrease of cell



Text-fig. 5. Comparison of time-courses of the shrinkage of cell area in pairs of crossed and uncrossed laminae; linear scales. The vertical bars represent 95 % confidence intervals for the percentage changes. Upper graph: ● = lamina 1, ○ = lamina 2; lower graph: ● = lamina 6, ○ = lamina 5.

area at 4 days, but at 8 days all show considerable shrinkage which exceeds that of the crossed laminae, being between 20 and 30 %. The difference at 8 days was confirmed for laminae 3 and 4 by measuring groups of fifty cells from an animal killed $7\frac{1}{2}$ days after eye enucleation; as shown in the graphs, there was close agreement of the two sets of observations. From 8 to 33 days the uncrossed laminae show a slight recovery in cell area, while in the crossed laminae there is a further marked shrinkage, of 30 %, between 16 and 33 days. The difference between the laminar groups at 33 days is a striking one, for it is well over 20 %. Unfortunately, however, its validity is a little uncertain, for in this animal the normal neurons were considerably larger in the crossed than in the uncrossed laminae. The fact that the nucleus and nucleolus have behaved differently in the two laminar groups in relation to cell area in this animal indicates a difference, but it is much less well established than that existing within the first fortnight. Text-fig. 5, which is plotted with linear scales, shows the time-course of changes in cell area for two pairs of laminae from opposite groups, for direct comparison.

Assessment of possible cell loss. No loss of neurons from the atrophied laminae, following transneuronal degeneration, could be demonstrated at 16 or at 91 days. At 129 days, which was the longest survival period, three of the atrophied laminae (1, 2 and 6) showed a slight decrease in the population of neurons within the sample volume studied, but this change was significant only in lamina 6. For all atrophied laminae together, however, the mean decrease in population was 7·8 %, and this, though significant, is a very small change.

DISCUSSION

The three most significant findings of the present study are: (1) the marked rapidity of onset of the transneuronal changes in the monkey; (2) the greater severity of the neuronal atrophy in the lateral geniculate nucleus of the monkey than in the cat and rabbit; and (3) a distinct difference in behaviour between the cells of the crossed and the uncrossed laminae which is in striking contrast with the similarity in behaviour of the cells of the three laminae in each group. An incidental finding which is of interest, although it is unrelated to the problem of transneuronal degeneration, is the observation that there does not appear to be any characteristic gradient of cell size from the ventral to the dorsal small-celled laminae.

The cells of the lateral geniculate nucleus of the monkey undergo essentially the same changes as have been described in the lateral geniculate nucleus of the cat and rabbit (Cook *et al.* 1951) and of man (Goldby, 1957). In general these changes consist of a reduction in cell size affecting all components of the cell, but particularly the cytoplasm, together with a diminution in the amount of Nissl material as assessed by the intensity of staining. From the evidence available at present, both for the lateral geniculate nucleus and for other sites, it may be concluded that in adult animals of all species transneuronal atrophy appears qualitatively to be the same. There are two possible qualifications, however, for Cook *et al.* (1951) described distinct vacuolation of the degenerating cells of the lateral geniculate nucleus of the cat and rabbit after 8–10 months, and Penman & Smith (1950) found that the medium-sized cells of the spinal nucleus of the trigeminal nerve in man had undergone swelling and chromatolysis four months after alcohol injection of the trigeminal ganglion.

In their paper on the termination of optic fibres in the lateral geniculate nucleus of the monkey, Glees & Le Gros Clark (1941) mention that transneuronal cell degeneration had occurred in one of their animals as early as 7 days after section of the optic nerve. The present study has confirmed this observation of the rapidity of onset, and has provided quantitative evidence of the severity of the cellular degeneration. Thus, as can be seen from the results summarized in Table 10, significant cell shrinkage occurred in the animal with the shortest survival period of this series (4 days), and the shrinkage progressed during the course of the next 1–2 months until the cross-sectional area of the cell was reduced to approximately 60 % of the normal. The degeneration, however, does not appear to be a uniform process; there are distinct differences in the rate at which it occurs in the cells of different laminae and in the extent to which the cytoplasm, nucleus and nucleolus of the cells are affected.

An analysis of the time-course suggests that in the monkey the degeneration occurs in three phases. There is an early rapid shrinkage of neurons in all six laminae which reaches its peak in the crossed laminae by 4 days and in the uncrossed laminae by 8 days. This early phase is followed by a period of relative stability lasting for approximately 2–3 weeks. After this the degeneration enters its third phase, which is characterized by a progressive shrinkage in mean cell area during the next 2 months. Thereafter the degenerative process appears either to have stabilized, or to have become considerably slower. Previous quantitative studies of transneuronal degeneration have shown nothing comparable to the early phase of shrinkage, and only in man has such a severe degree of atrophy been described (Goldby, 1957). In their detailed study of this type of degeneration in the lateral geniculate nucleus of the cat, Cook *et al.* (1951) found no evidence of cell shrinkage earlier than 63 days after eye enucleation, and thereafter the degeneration as estimated by cell size remained more or less constant until 313 days, which was their longest survival period. In the rabbit they found no consistent signs of degeneration until 5–6 months after enucleation of the eye.

That the cytoplasm, nucleus and nucleolus do not behave uniformly during transneuronal degeneration is also clearly seen in Text-fig. 3. From this figure it is apparent that at nearly every interval studied the degree of shrinkage of the cytoplasm exceeds that of the nucleus. Although the nucleolar measurements are somewhat less accurate, the evidence available suggests that during transneuronal degeneration the changes in the nucleolus follow those in the nucleus more closely than they follow the cytoplasmic changes. Regarding the severity of the cellular degeneration, however, there appear to be distinct differences between species in the degree of atrophy ultimately reached. In the lateral geniculate nucleus of the cat Cook *et al.* (1951) found the shrinkage to be of the order of 20–30 %; in the lateral geniculate nucleus of the rabbit they describe the shrinkage as being slightly less, although they give no figures for this species. In the macaque, and in man, the end-result appears to be more severe; the present observations have shown that after 129 days the cell shrinkage in all laminae is of the order of 30–40 %, which is only slightly less than the shrinkage found by Goldby (1957) in a human lateral geniculate nucleus at least 36 years after eye enucleation. Not only the cell area, but also the nuclear and nucleolar areas, show more marked changes in the monkey than in the cat (20–30 %, as compared with approximately 15 %).

In the lateral geniculate nucleus of the Primates, moreover, there is after relatively long survival periods a difference in the degree of atrophy between the cells of the large-celled and small-celled laminae. This difference has been noted by Le Gros Clark (1941) and Polyak (1957) in the macaque, and in man by Hechst (1933) and Goldby (1957); Goldby measured the actual degrees of shrinkage of these cells in his human case. It is also well known that these cells behave differently after visual cortex ablation (Brouwer, 1930; Walker, 1935; Powell, 1952). The finding in the present study that during the first 4 months after eye enucleation there is no essential difference either in the time-course or in the severity of transneuronal degeneration between the large- and small-celled laminae can probably be explained by the fact that these survival periods were comparatively short. That the observed difference in behaviour between the large and the small cells is not merely a function of the size of the cell soma, but is due to some more fundamental difference in the neuron or in the organization of its connexions, is suggested by recent evidence from the primary auditory nuclei that neurons of markedly different size may undergo transneuronal degeneration at the same rate and to the same degree (Powell & Erulkar, unpublished).

Cell loss. Despite the rapidity and severity of the cellular changes in transneuronal degeneration found in the present material there is no good evidence of any appreciable cell loss during the first 4 months after enucleation of the eye. In only one animal (4 months' survival) was there any evidence of cell loss; here there was a slight loss in three laminae, but only in lamina 6 was it statistically significant. This finding may represent the initial stage of a slow but progressive cell loss.

The question whether or not cell loss occurs in transneuronal degeneration is of considerable interest. In Primates severe cell loss has been described in the lateral geniculate nucleus following eye removal. Polyak (1957) found 'complete disappearance of most of the nerve cells and a considerable increase in the neuroglia' in the affected laminae of the lateral geniculate nucleus of a macaque in which the retina had been scooped out at birth, and which was then allowed to survive for 2 years. In the human case described by Goldby (1957) the atrophic laminae of the lateral geniculate nucleus showed a cell loss of approximately 50 % many years after removal of the eye as a result of a war injury. Similarly, Penman & Smith (1950) described 'a definite reduction in the number of the neurons' in the main sensory nucleus of the trigeminal nerve of man, and in the spinal nucleus 'clumps of microglial cells are seen suggesting neuronophagia', 4 months after injection of alcohol into the trigeminal ganglion. On the other hand, in the lateral geniculate nucleus of the cat and rabbit Cook *et al.* (1951) found 'no evidence of neuronophagia' up to 313 days. Whether the increasing vacuolation which they found in the animals with the longest survival periods is an indication that the degenerative process might progress to cell loss is unknown. In the ventral cochlear nucleus and lateral superior olive of the cat, however, detailed counts throughout the nuclei showed no evidence of cell loss 319 days after destruction of the cochlear nerve (Powell & Erulkar, unpublished). In marked contrast to these results in adult cats, Torvik (1956) has described almost complete cell loss in restricted areas of the pontine and inferior olivary nuclei as early as 5–8 days after lesions of the cerebral hemispheres and brain stem in young kittens (8–20 days old).

From these observations it seems clear that the degree of cell loss occurring in any given species is a function at least of the age of the animal at the time of operation and of the duration of the post-operative survival period. It would be of interest to test experimentally the validity of this deduction by a study of the time-course and severity of transneuronal degeneration in the lateral geniculate and cochlear nuclei of young rabbits and kittens, or in the pontine and inferior olivary nuclei of mature cats, and in the lateral geniculate nucleus of monkeys after longer survival periods. The fact that in transneuronal degeneration in adults appreciable cell loss has only been described in man might suggest that species is also a determining factor.

Differences in behaviour between crossed and uncrossed laminae. A point of considerable theoretical interest which has emerged from this study is the finding that the neurons of the crossed and uncrossed laminae show differences both in the severity and time of onset of the initial phase of transneuronal atrophy. Thus the cells of the crossed laminae have virtually reached the end of this phase by 4 days, while the neurons of the uncrossed laminae show little morphological evidence of atrophy until somewhat later. At 8 days, however, the degree of shrinkage which they have undergone is greater than that seen in the crossed laminae, and this observation has been confirmed in one animal which was allowed to survive for 7½ days in which the severity of the cell shrinkage was essentially the same. In contrast to this difference between the cells of the crossed and uncrossed laminae, the neurons of all three laminae of either group consistently showed the same pattern of degeneration, except during the first few days when there was a greater degree of atrophy of the cells of the dorsal small-celled laminae (i.e. lamina 5 of the uncrossed and lamina 6 of the crossed group).

Qualitatively there appear to be no morphological differences between the neurons of the crossed and uncrossed laminae in the normal lateral geniculate nucleus. The only known quantitative difference is the finding that the mean cell area of the uncrossed small-celled laminae exceeds that of the crossed, but in the large-celled laminae this relationship is reversed. No differences have been described either in the number or in the form of the synaptic endings in the crossed and uncrossed laminae. Indeed, when discussing their experimental study of the termination of the optic nerve fibres in the monkey, Glees & Le Gros Clark (1941) state that 'it seems probable that no cell in any of the laminae receives more than one bouton', and from their description it appears that all synaptic terminals are of the axosomatic type. In this connexion, however, it may be apposite to recall that the appearance of the terminal degeneration at 3 days as described by these authors differed in the crossed and uncrossed laminae. In laminae 2, 3, and 5 they found the terminal boutons to be 'in many instances thickened, somewhat enlarged, and stained more densely than in normal preparations'. In the crossed laminae 1, 4 and 6 similar changes were observed, but in addition they state that 'the interior of the ring-like boutons in these laminae is often found to be filled with a dense, opaque mass which on close scrutiny appears to be composed of a very fine neurofibrillar network. In other instances the boutons have become converted into small, solid, black end-bulbs.' From a knowledge of the time-course of bouton degeneration it seems justifiable to infer from this description that the terminal degeneration had proceeded further in the crossed than in the uncrossed laminae.

Two other differences in the afferent fibres which may be of importance in this connexion are the greater length of the crossed fibres and the relative diameters of the crossed and uncrossed components of the optic tract. It would be of interest to know, for example, whether in Primates there is a difference in mean fibre diameter between the crossed and uncrossed optic tract fibres comparable to that found in the cat by Bishop, Jeremy & Lance (1953), and whether optic nerve fibres of different diameters have differing rates of degeneration such as van Crevel (1958) has shown in the cat. In view of these known differences in the afferent fibres of the cat, it would be of interest, also, to determine whether transneuronal degeneration differs in its time of onset in the crossed and uncrossed laminae of this species.

General observations on the process of transneuronal degeneration. Despite all the work which has been done in recent years, the factors which determine whether or not a cell will degenerate after removal of some or all of its afferent connexions are not clearly known; but the severity and time-course of the degeneration appear to depend upon at least three main variables. The first of these is the age of the animal at the time of operation. All other neuronal degenerations which have been adequately studied so far have proved to be more severe in younger animals, and the interesting work of Torvik (1956) has indicated that transneuronal atrophy is no exception to this rule. His work has shown that transneuronal changes are both more severe and more rapid of onset in young animals than in adults; indeed, it is probable that this type of degeneration would be found to occur more widely if young animals were used in such experimental studies.

The second factor of importance is the species of the animal used. Its effect upon the time-course is illustrated by the marked difference in the time of appearance of transneuronal changes in the monkey, cat and rabbit; in the monkey definite changes become apparent within a few days of deafferentation, but in the cat and rabbit the changes can only be seen after about 2 months. In consequence of such differences of time-course, it is obvious that the duration of the survival period required to demonstrate unequivocal transneuronal degeneration may vary markedly from species to species. The differences in the severity of neuronal changes between cat, rabbit, monkey and man, at the longest survival periods for which data are available, have been mentioned above; it would appear for the first two species at least that, once transneuronal degeneration is established, further survival (up to 1 year in the cat) makes little difference to the degree of cellular shrinkage. On the other hand, it is not certain whether cell loss would occur at some later stage, and it would be of interest to know if after very long survival periods some slow, but progressive change, such as the increasing vacuolation which was described by Cook *et al.* (1951), would lead to cell loss comparable to that found by Goldby (1957) in the human lateral geniculate nucleus. The cell loss in the human lateral geniculate is not necessarily dependent upon the long post-operative survival, however, in view of the observations of Penman & Smith (1950) on the trigeminal nuclei.

The differences in transneuronal degeneration of the visual system in these four species are sufficiently great to suggest that there is some fundamental difference in the lateral geniculate neurons themselves, or in their afferent connexions. It is impossible at present to decide which of these factors is the more critical, as there are known differences in each. For example, the lateral geniculate nucleus shows distinct differences in behaviour after visual cortex ablation: in Primates all the cells undergo complete degeneration (Poljak, 1933), but in the cat a definite proportion of cells remains (Minkowski, 1913). Whether these persisting cells are principal neurons which have survived axonal section, or represent the short-axon cells described by O'Leary (1940), is not known. Differences have also been described in the afferent terminals: Glees & Le Gros Clark (1941) found only a single axosomatic bouton on each lateral geniculate cell of the monkey, but in the cat and rabbit Glees (1941, 1942) has described multiple endings of both the axosomatic and axodendritic types.

The third variable which may be considered is the nuclear formation in which transneuronal degeneration is studied. Here the evidence available suggests that in adult animals of the same species, transneuronal degeneration will probably be found to show very much the same severity and time-course in all regions in which it occurs. In an unpublished series of observations on the cochlear nuclei and lateral superior olive of the cat, the transneuronal degeneration following section of the cochlear nerve has been found to show a time-course and a degree of neuronal shrinkage virtually identical with those described for the lateral geniculate nucleus in this species. In a study of the superior cervical ganglion of the rabbit after preganglionic nerve section, Hamlyn (1954) found cell shrinkage of up to 18% after 3–4 months, which is probably of the same order as that described qualitatively by Cook *et al.* (1951) in the rabbit lateral geniculate, though occurring slightly earlier. It would obviously be of interest to obtain quantitative data on transneuronal degeneration in the superior cervical ganglion of the cat and monkey, and in the auditory nuclei of the rabbit and monkey. Structural or functional differences between groups of neurons may, however, produce some differences in transneuronal atrophy, as indicated, for example, by the differences in the time-course of the changes between crossed and uncrossed laminae of the monkey lateral geniculate nucleus, and in ultimate severity of the degeneration between the large and small neurons of the lateral geniculate nucleus in man and in the monkey.

The question of the region affected by transneuronal degeneration in any given species may not be important as far as the time-course and severity of the degeneration are concerned; the site certainly is significant, however, in determining whether or not transneuronal degeneration does occur after interruption of the afferent connexions. To take but one example, the absence of transneuronal degeneration in the ventral posterior nucleus of the thalamus of the monkey after section of the medial fillet is in striking contrast to the unequivocal degeneration found in the lateral geniculate nucleus of the same animal (Glees & Le Gros Clark, 1941). The factor critical in determining the occurrence of transneuronal degeneration after deafferentation of neurons is almost certainly the extent to which afferents have been destroyed. The absence of transneuronal degeneration in the ventral thalamic nucleus is probably explicable on this basis, and Cook *et al.* (1951) have suggested the same explanation for its absence in the anterior horn cells of the spinal cord following ablation of the motor cortex and dorsal root section. The precise proportion of afferents which has to be destroyed for transneuronal degeneration to occur is unknown, and may vary from nucleus to nucleus. The only evidence bearing on this point is Torvik's (1956) statement for the young kitten that 'heavy degeneration is observed even though at least one-half of the afferents are intact (as evidenced by the bilateral changes in the olive in unilateral lesions)'.

SUMMARY

1. A quantitative study has been made of transneuronal atrophy in the lateral geniculate body of *Macaca mulatta*. One eye was enucleated from each of twelve mature monkeys, and they were allowed to survive for periods varying from 4 days to 4 months. The neurons of the atrophied laminae in each geniculate were compared with those in the normal laminae of the opposite geniculate innervated from the intact eye. One unoperated monkey was used as an additional control.

2. The appearance of the normal neurons is described. A small percentage of normal neurons had two nucleoli; this was commoner in the small-celled laminae (3–6) than in the large-celled laminae (1 and 2). Measurement of normal neurons has shown that regular gradients of cell area from lamina to lamina are uncommon, and that the neurons of any given lamina cannot be grouped into more than one population, when assessed according to the size of their cell bodies.

3. During transneuronal atrophy, the neurons shrink and the staining intensity of their Nissl substance diminishes. In the first few months, the cytoplasm shrinks proportionately more than the nucleus and nucleolus. At 4 months there was an increase in the incidence of neurons with two nucleoli.

4. The shrinkage of neurons has been found to show two phases, an early rapid phase which affects the laminae receiving crossed fibres (1, 4 and 6) earlier than those receiving uncrossed fibres (2, 3 and 5), and which is succeeded by a period of relative stability, and a later phase in which there is no consistent difference in behaviour between the groups of laminae. Within each group, the neurons of all three laminae of each crossed or uncrossed set behaved rather similarly, except during the first few days when it was the dorsal small-celled lamina (5 or 6) which was the most affected.

5. The early rapid shrinkage of neurons reached its peak in the crossed laminae by 4 days, and in the uncrossed laminae by 8 days; this shrinkage was more profound (24–30 % of control area) in the uncrossed than in the crossed laminae (9–17 % of control area). This phase coincided with an onset of apparent gliosis in the atrophic laminae.

6. The later phase of atrophy began towards the end of the first month, or during the second month, and was possibly earlier in the crossed laminae. By 4 months, further shrinkage of neurons appeared to have ceased, at least in the uncrossed laminae; the mean area of the atrophied neurons was now about 60 % of the mean area of the control neurons in the opposite lateral geniculate.

7. No indication of loss of neurons was found until 4 months after eye enucleation, when there appeared to be a slight but significant loss of neurons in lamina 6.

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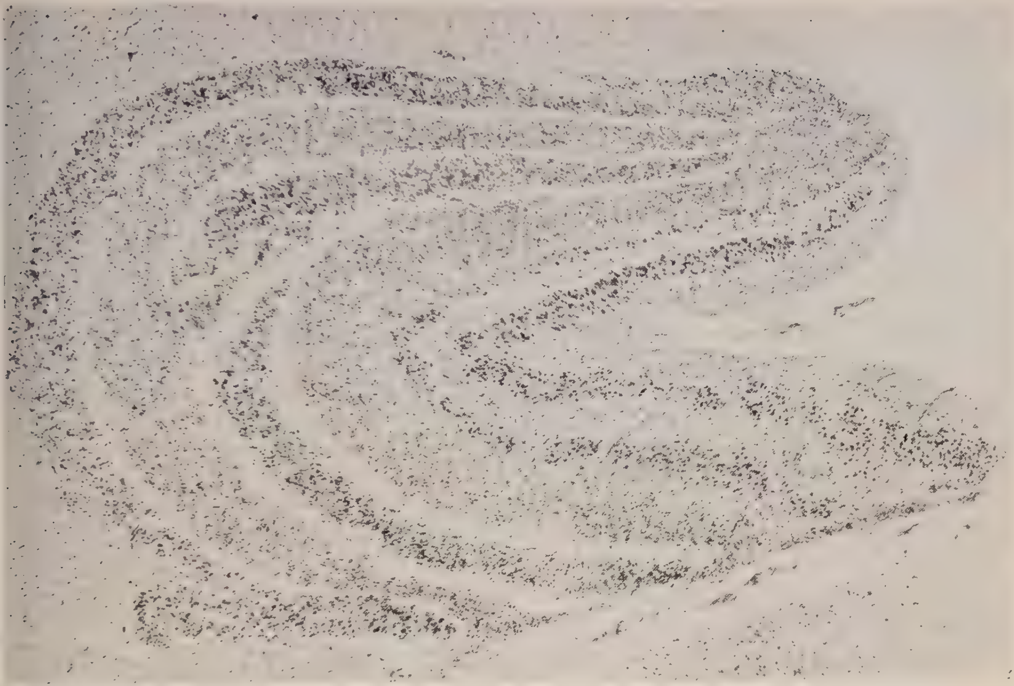
EXPLANATION OF PLATES

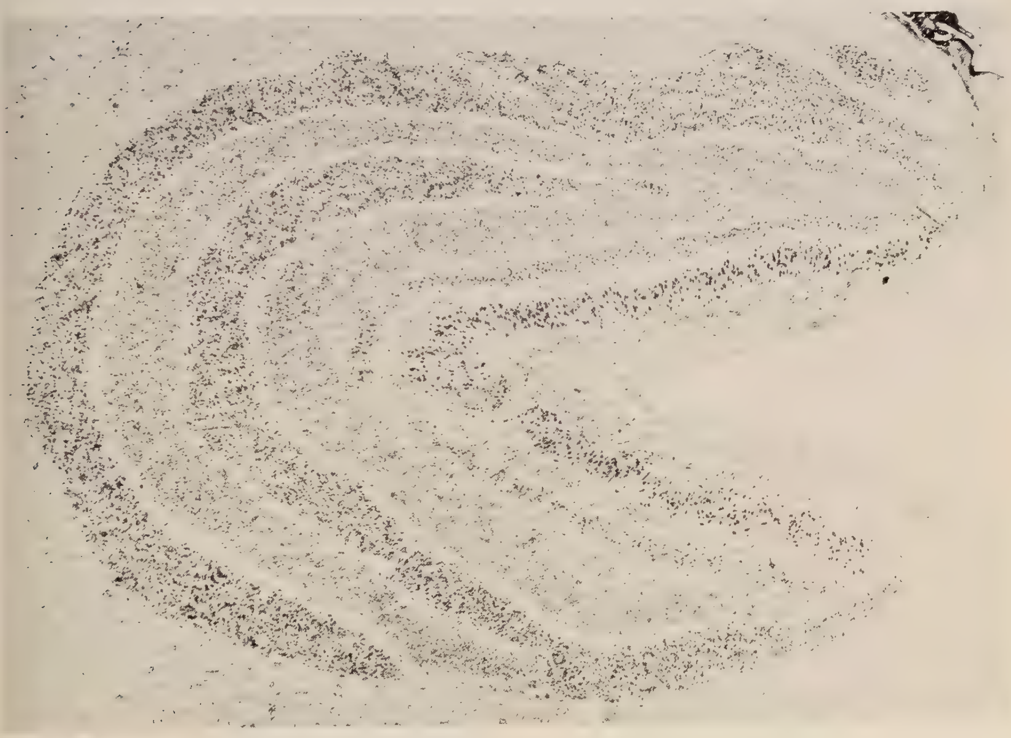
All sections were 25μ in thickness and were stained with thionin.

Plate 1

Fig. 1. Coronal section from the posterior half of the left lateral geniculate nucleus of a macaque, 6 days after enucleation of the left eye. There is distinct atrophy of neurons in the uncrossed laminae (large-celled lamina 2, small-celled laminae 3 and 5). The crossed laminae (1, 4 and 6) are normal. × 24.

Fig. 2. Coronal section from the posterior half of the left lateral geniculate nucleus of a macaque, 91 days after enucleation of the left eye. The neurons of the three uncrossed laminae are severely shrunken. × 24.





MATTHEWS, COWAN AND POWELL—TRANSNEURONAL CELL DEGENERATION

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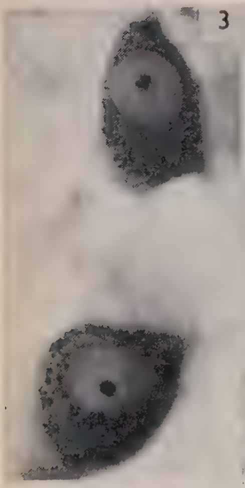
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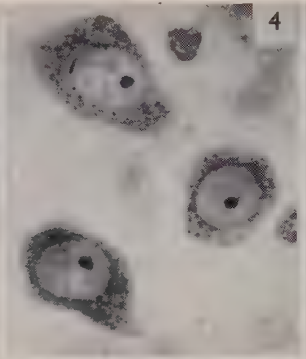
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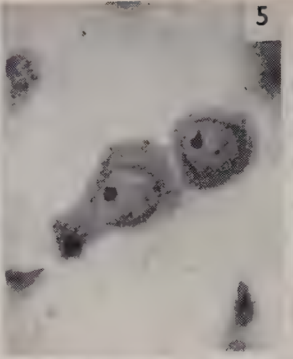
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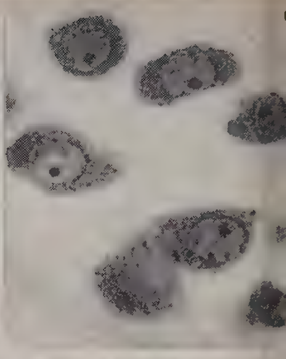

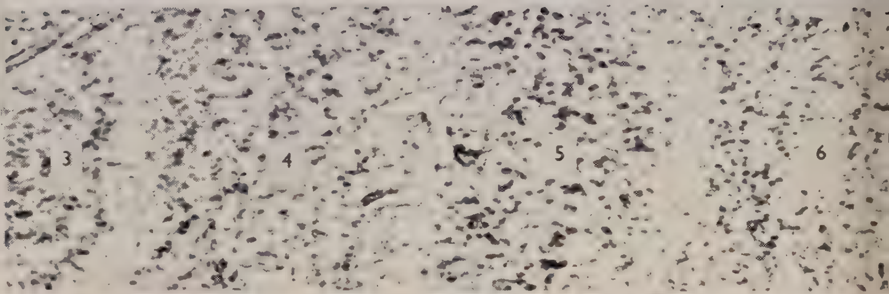

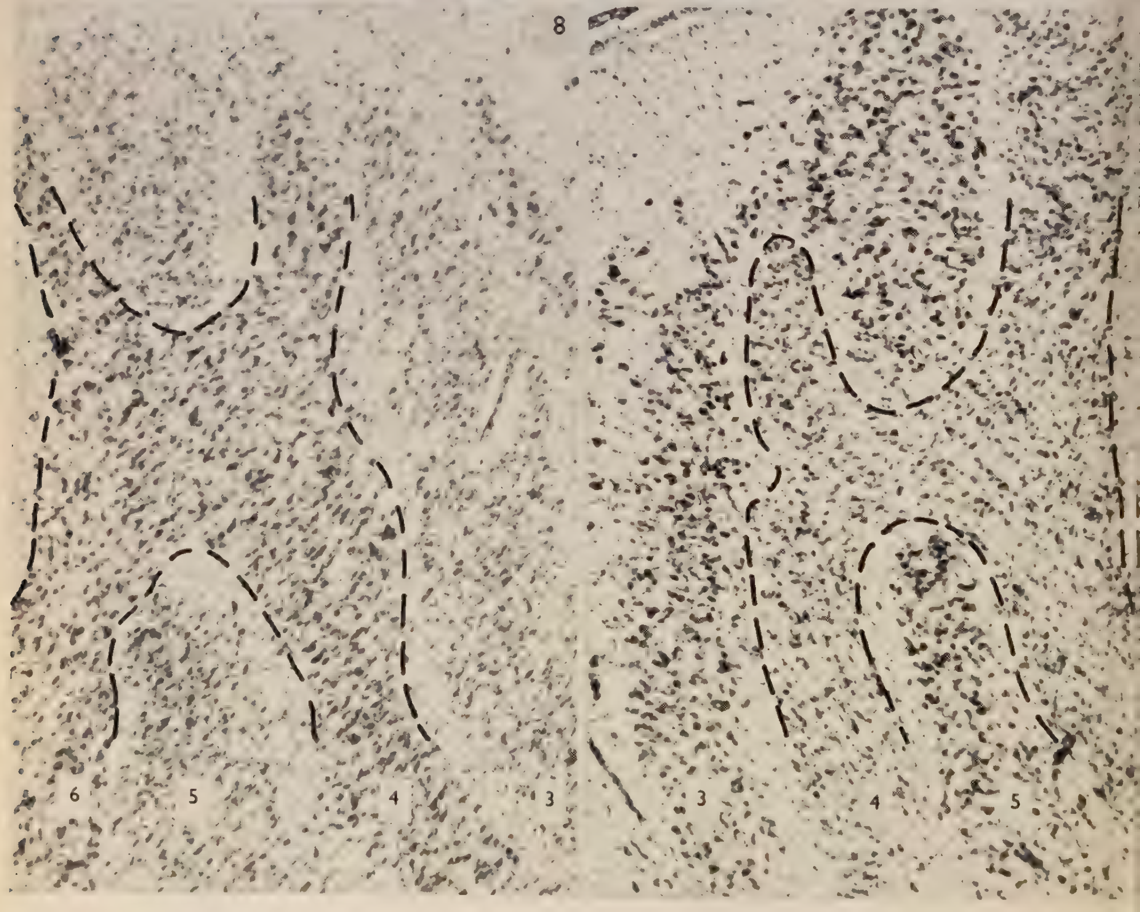
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MATTHEWS, COWAN AND POWELL—TRANSNEURONAL CELL DEGENERATION

PLATE 2

Figs. 3 and 4. Normal (Fig. 3) and atrophied (Fig. 4) neurons from lamina 1 of the lateral geniculate nuclei of a macaque, 129 days after removal of one eye. × 1000.

Figs. 5 and 6. Normal (Fig. 5) and atrophied (Fig. 6) neurons from lamina 5 of the lateral geniculate nuclei of a macaque, 129 days after removal of one eye (same animal as Figs. 3 and 4). × 1000.

Fig. 7. Part of a coronal section from the left lateral geniculate nucleus of a macaque, 4 days after enucleation of the right eye. This strip passes perpendicularly across the small-celled laminae 3–6, which are numbered, and shows the pallor and shrinkage of neurons in the crossed laminae, 4 and 6. The uncrossed laminae on this side are normal. × 72.

Figs. 8 and 9. Parts of coronal sections at corresponding levels of the two lateral geniculate nuclei of a macaque, 8 days after enucleation of the right eye. Portions of the lateral 'limbs' are shown, and the small-celled laminae are numbered. On each side, a band of cells connects laminae 4 and 6 at this level, interrupting lamina 5; this is indicated by a dotted line. These photographs are complementary, showing the shrinkage of the uncrossed laminae in the right lateral geniculate (Fig. 8) and of the crossed laminae in the left lateral geniculate (Fig. 9). × 72.

WOUND CONTRACTION IN RABBIT SKIN, STUDIED BY SPLINTING THE WOUND MARGINS

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INTRODUCTION

During the last five years there has been a much increased interest in the contraction of skin wounds, the remarkable process by which the edges of the wound are drawn together, reducing the size of the resulting scar to much less than that of the original defect. For a long time it had been widely assumed that the force motivating this contraction came from the newly formed collagen within the wound. Abercrombie & James (1955) and Abercrombie, Flint & James (1954, 1956) presented some evidence that this was unlikely, but neither their evidence nor the alternative hypothesis they proposed, that the force came from the cells of the granulation tissue, has found much favour with subsequent workers. The collagen dogma has nevertheless recently lost something of its hold, and at least three further hypotheses have been introduced (van den Brenk, 1956; Watts, Grillo & Gross, 1958; Cuthbertson, 1959). The subject, previously suffering from one all too generally accepted hypothesis, is now beginning to suffer from the opposite extreme.

In the circumstances, more experimental data are evidently required. It seemed to us that useful information could be got by hindering contraction and then suddenly releasing the constraint; thereby we hoped to obtain successively a slowing and an exaggeration of the speed of the process. Lindquist (1946) used extensively a technique of splinting wounds with adhesive plaster; but it was particularly a note by Marshak (1945), who prevented contraction by a plastic dressing, and on release obtained rapid contraction of the wound with thickening of the granulation tissue, which suggested the value of a splinting technique.

MATERIAL AND METHOD

Adult male white rabbits (mean weight 2·44 kg., range 2·10–3·50 kg., standard deviation 0·78 kg.) from breeders' stock were used. They were anaesthetized with intravenous Nembutal reinforced with open ethyl ether, and hair was removed from the sides of the chest with electric clippers. The skin of each side of the thorax was then tattooed in the following manner. First, the animal was tied with its forelimbs in extension. With indian ink and a mapping pen, a square of approximately 2 × 2 cm. was marked by sixteen symmetrically arranged dots. A no. 22 straight cutting needle, loaded with indian ink and held in a small engineers' chuck, was then inserted through each dot into the underlying dermis, and rotated before withdrawal. The sides of the square thus tattooed were parallel with the cranio-caudal and dorso-ventral axes.

Immediately afterwards incisions were made aseptically along the square marked out by the inner margins of the tattoo dots (see Abercrombie *et al.* 1954) and were extended down to the panniculus. The island of skin thus freed was dissected off the panniculus, and preserved for subsequent analysis.

The outline of the wound was next traced on to the central part of a 5 × 5 cm. square of $\frac{1}{16}$ in. thick Perspex, bent to approximate conformity with the dorso-ventral curvature of the thoracic wall. The traced area was cut out from the Perspex with a fretsaw, and the splint thus formed, with central hole matching the wound, was glued to the skin surrounding the wound with a collodion adhesive (a diluted version of that of Cannon & Longmire, 1952, due to Dr L. Brent, to whom we are most grateful). The wound was sprayed with penicillin and sulphathiazole powder, and dressed with tulle gras and sterile gauze.

For measurements of the wound area, tattoos were traced on to cellophane before operation, after excision of the skin, and again after the splint was applied.

The contralateral side of the chest was wounded, splinted, traced, and dressed in a similar manner, and finally the animal was bandaged and a light plaster cast applied. 200,000 units of procaine penicillin were given intramuscularly.

With the exception of the first four of the total of fifteen animals used, dressings were removed under anaesthesia at 3–4 days and the tattoos traced. The granulation tissue in the bed of the wound was then tattooed with eight dots placed 1–2 mm. from the skin margin, opposite the skin tattoos marking the four corners and mid-points of the original square. Both the original and the new tattoos were traced again on cellophane, and the wounds dressed as before.

At 10 days, again under anaesthesia, the tattoos were traced once more. The thirty wounds available from the fifteen animals used were divided randomly into three equal groups for subsequent treatment. In group A, the splint was removed at 10 days, the tattoo traced half an hour later, and the granulations within the wound biopsied at 12 days. In group B, granulations were first biopsied and the splint then removed, both at 10 days (one measurement of a wound in this group was lost, so the area data of this group are given for only nine wounds). In group C, wound and splint were left undisturbed after tracing at 10 days, and the granulations biopsied and the splint then removed at 12 days.

In all groups the technique of biopsy of the granulations was similar. The tattoos were first traced. The granulations were then marked with a ring of indian ink, so arranged as to clear the surrounding skin edges by about 1–2 mm., to avoid all possibility of contamination with the original skin. The ink ring was first traced on cellophane, and an incision then carried through it vertically down to the panniculus. Great care was taken not to cut into the panniculus. Thirty minutes later a further tracing was taken showing the central island isolated by the incision, the corresponding peripheral margin of the incision into the granulations, and the tattoos. The island of granulation tissue was then excised for analysis, and the splints were removed from the wounds of groups B and C around which they were still in position: the tracing was repeated on these after a further 30 min. and the animal redressed.

Further tracings were made at 2 and 5 days after biopsy.

From the tracings on cellophane further tracings were made on to paper of standard thickness. These areas were cut out with scissors and weighed, and results calculated in cm.^{2} (Hamlyn, 1954).

Chemical methods. Both skin and granulation tissue were weighed in stoppered bottles, dried to constant weight at 102° C., and stored until required at −10° C.

For estimation of collagen and nitrogen, tissue was first treated with 10% trichloroacetic acid (TCA) at 90° C. for 30 min. (Fitch, Harkness & Harkness, 1955). It was then homogenized in a Potter–Elvehjem type homogenizer, heated for a further 30 min. at 90° C. and finally made to volume with 10% TCA. Two 1 ml. volumes of the product were removed for nitrogen estimation (Ma & Zuazaga, 1942) and the remaining homogenate centrifuged at 2500 r.p.m. for 10 min. Two 1 ml. volumes of the supernatant were taken for the estimation of hydroxyproline (as an index of collagen content) by the technique of Neuman & Logan (1950) as modified by Martin & Axelrod (1953). After colour development absorption measurements were made at 560 mμ with a spectrophotometer, and the hydroxyproline contents established from standard curves.

Twenty-two of the thirty granulation biopsy specimens were divided into two approximately equal parts before weighing. One was treated as already described. The second was analysed for deoxyribose nucleic acid (DNA) by the method of Schneider (1945).

RESULTS

The wounds during the first 10 *days*

During this period the wounds of all three groups were treated essentially alike, and the measurements of their behaviour can consequently be pooled. The area described by joining up the centres of the tattoo marks ('skin tattoo area') before operation had a mean size of 4·20 ± 0·08 cm.^{2}. The excision within the tattoo marks led to an immediate expansion of this area to 6·31 ± 0·15 cm.^{2}. This expansion is the expression of the tension normally present in skin. The splint was then fitted, reducing the mean size of the area to 6·06 ± 0·12 cm.^{2} (standard deviation 0·66, range 4·4–7·9) and this therefore was the size at which the process of healing started ('initial size', tabulated separately for the different groups in Table 1).

Table 1. *Mean sizes, in cm.*^{2}*, with standard errors, of areas marked by skin tattoos*

In group A, the splint was removed after the measurement at 10 days. In group B, most of the granulation tissue was excised and the splint removed after the measurement at 10 days. In group C neither splint nor granulation tissue was removed.

Group	No. of specimens	Initial size	10-day size	12-day size
A	10	6·15 ± 0·14	5·47 ± 0·18	2·55 ± 0·14
B	9	5·70 ± 0·22	4·98 ± 0·24	3·43 ± 0·11
C	10	6·25 ± 0·24	5·48 ± 0·37	4·75 ± 0·34

During the next 10 days, in spite of the presence of the splint, there was a small but significant contraction of the mean skin tattoo area to 5·35 ± 0·16 cm.^{2}. The skin margin around the wound, bearing the tattoo marks, protrudes slightly into the hole in the middle of the splint and so is not immobilized and can be drawn inwards. The centres of the tattoo marks only have to move inwards about three-quarters of a mm. to produce this amount of contraction, though the cut edge of the skin is drawn inwards farther than this. These wounds therefore provide information about what happens during hindered rather than completely suppressed contraction. It may be mentioned that three unsplinted wounds of the same size and shape as the splinted ones (initial size $6{\cdot}25 \pm 0{\cdot}45$ cm.^{2}) had by 10 days contracted to $2{\cdot}34 \pm 0{\cdot}26$ cm.^{2}.

In twenty-two of the wounds, the dressing was removed at between 3 and 5 days. and tattoo marks put into the granulation tissue. The mean size of the skin tattoo areas of these twenty-two wounds at this time was $5{\cdot}84 \pm 0{\cdot}11$ cm.^{2}, but this is not a significant decrease from their original size (the decrease was $0{\cdot}07 \pm 0{\cdot}10$ cm.^{2}). The size of the central granulation area enclosed by the granulation tattoos ('granulation tattoo area') was $3{\cdot}09 \pm 0{\cdot}15$ cm.^{2} (tabulated separately for the different groups in Table 2). By 10 days after operation the mean granulation tattoo area was only $1{\cdot}86 \pm 0{\cdot}09$ cm.^{2} and had therefore lost $1{\cdot}23 \pm 0{\cdot}11$ cm.^{2}, a shrinkage much exceeding that of the whole wound. Consequently the rim of granulation tissue and normal skin outside the central granulation tattoo area but inside the border of the skin tattoo area had expanded by $0{\cdot}52 \pm 0{\cdot}13$ cm.^{2}; perhaps largely or wholly because of the stretching of that width of the skin margin protruding from the splint represented by the radius of the skin tattoo marks. The diminution of the central granulation tattoo area makes it clear that the granulations are contracting down in area and are not simply being over-ridden by the surrounding skin.

Table 2. *Mean sizes, in cm.^{2}, with standard errors, of areas marked by tattoos made on the granulation tissue at 3 or 4 days after wounding*

In group A, the splint was removed after the measurement at 10 days. In group B, most of the granulation tissue was excised and the splint removed after the measurement at 10 days, so there are no measurements at 12 days. In group C neither splint nor granulation tissue was removed.

Group	No. of specimens	Initial (3 to 4-day) size	10-day size	12-day size
A	6	$3{\cdot}06 \pm 0{\cdot}11$	$2{\cdot}03 \pm 0{\cdot}09$	$0{\cdot}87 \pm 0{\cdot}12$
B	10	$3{\cdot}02 \pm 0{\cdot}26$	$1{\cdot}77 \pm 0{\cdot}13$	
C	6	$3{\cdot}24 \pm 0{\cdot}36$	$1{\cdot}85 \pm 0{\cdot}25$	$1{\cdot}27 \pm 0{\cdot}20$

Different treatments of wounds between 10 *and* 12 *days*

The wounds were now divided into three groups, one of nine and two of ten specimens. These groups have been found by analysis of variance not to differ significantly (at the 10 % level) in mean skin tattoo area before operation, after operation or after splinting; nor in the contraction undergone up to 10 days, nor in size at 10 days; nor in size of the granulation tattoo area, nor of the skin tattoo area at 3–5 days when the granulation tattoos were applied, nor in shrinkage of the granulation tattoo area. In all respects therefore they were adequately randomized. The three groups were treated differently at 10 days, and the effect of this was determined at 12 days. In group A, the dressings were removed at 10 days, the skin tattoo area was measured (see Table 1), then the splint was removed and after 30 min. observation the wound was redressed, with no excision. At 12 days the wound was examined again, and it was found to have lost $2{\cdot}92 \pm 0{\cdot}16$ cm.^{2} of its area, that is, to have contracted down to less than half the size it had at 10 days. These wounds, with a mean size of $2{\cdot}55 \pm 0{\cdot}14$ cm^2, were still a little larger than wounds which had never been splinted but had otherwise been similarly treated. Five such wounds, with a mean initial size of $6{\cdot}05 \pm 0{\cdot}35$ cm.^{2}, had by 12 days become $1{\cdot}73 \pm 0{\cdot}07$ cm.^{2} in area.

In group B, when the skin tattoo area had been measured at 10 days (see Table 1) the central part of the granulations (an area rather bigger than the granulation tattoo area, amounting to about two-thirds of the total area of granulation tissue) was excised, and shortly afterwards the splint removed. At 12 days the mean tattoo area had lost $1{\cdot}55 \pm 0{\cdot}20$ cm.^{2}, significantly less than the loss in group A (t 5·4, D.F. 17, $P < 0{\cdot}001$).

In group C, when the skin tattoo area had been measured at 10 days (see Table 1) it was simply redressed, with the splint still in place and without excision. The wounds of this group had lost least area of the three groups between 10 and 12 days, a mean of $0{\cdot}72 \pm 0{\cdot}14$ cm.^{2}. This is significantly less than group B (t 3·43, D.F. 17, $0{\cdot}01 > P > 0{\cdot}001$). That the loss in area approximately equalled that which had occurred during the whole initial period of 10 days is probably to be explained by diminishing adherence of the splint to the skin; by 10 days merely removing the dressing was a disturbance sufficient to cause some escape of the wound margin from the splint.

In groups A and C, in which no excision was made at 10 days, the behaviour of the granulation tattoo area could be followed; this was done on a sample of the wounds (Table 2). In group A (6 specimens) which was desplinted at 10 days, the granulation tattoo area lost $1{\cdot}16 \pm 0{\cdot}11$ cm.^{2}, the rim around this $1{\cdot}51 \pm 0{\cdot}10$ cm.^{2}. This peripheral rim had actually expanded during the preceding splinted period (up to 10 days) and its contraction when released from the splint was particularly sharp. In group C (6 specimens), which was left splinted but nevertheless contracted faster than during the previous 10 days, the central granulation area lost $0{\cdot}58 \pm 0{\cdot}09$, while the peripheral rim did not change significantly (mean contraction $0{\cdot}10 \pm 0{\cdot}13$ cm.^{2}); contraction was therefore confined to the central granulation tattoo area, as during the first 10 days.

Immediate effect of splint removal

In group A the splint was removed from the intact wounds at 10 days. In groups B and C, the splint was removed from wounds a large proportion of whose granulation tissue had been excised 30 min. before; in group B this was done at 10 days, in group C at 12 days. We now consider the immediate effects (i.e. those within the succeeding 30 min.) when the tension of the splint is relieved in wounds differing in these respects.

In the intact wounds of group A, the effect of splint removal was a sudden contraction, the skin tattoo areas losing $1{\cdot}43 \pm 0{\cdot}12$ cm.^{2} (10 specimens); half the total contraction already mentioned as occurring in the 2 days following desplinting occurred therefore in the first half hour. This contraction was accompanied by a bulging of the granulation tissue above the skin surface, as Marshak (1945) found in similar circumstances. The central granulation tattoo area diminished by less than half the area lost in the 2 days, its contraction being $0{\cdot}46 \pm 0{\cdot}05$ cm.^{2} (6 specimens); while the rim area lost $1{\cdot}00 \pm 0{\cdot}18$ cm.^{2} (6 specimens), a good deal more than half.

Group B contracted rather more than group C when the splint was removed (group B, 10 specimens, $0{\cdot}72 \pm {\cdot}015$; group C, 6 specimens, $0{\cdot}39 \pm 0{\cdot}13$). The difference is not however significant, and the biopsied wounds of these two groups can be pooled for comparison with the intact wounds of group A. Their mean contraction is $0{\cdot}59 \pm 0{\cdot}11$ cm.^{2}, far less than that of group A (t 5·1, D.F. 24, $P < 0{\cdot}001$). On a small sample of groups B and C the area of the central space left by the excision, and of the remaining peripheral rim, was measured during the 30 minutes after removal of the splint. Both contracted, more in group B than in group C.

Immediate effect of incision into granulation tissue

In groups B and C incisions were made at 10 and 12 days respectively in the granulation tissue, still held by the splint, in such a way as to isolate, down to the panniculus carnosus, a central area of granulations of mean area $2{\cdot}09 \pm 0{\cdot}11$ cm.^{2} (its area did not differ significantly between the two groups). This was a larger area than that of the granulation tattoos; it represented 43 % of the total skin tattoo area ($4{\cdot}86 \pm 0{\cdot}21$ cm.^{2}) and at least two-thirds of the total area of granulation tissue. In group A similar incisions were made, but they were done 2 days after removal of the splint (which was done at 10 days), when the wound had already contracted considerably. The isolated central area was $1{\cdot}14 \pm 0{\cdot}10$ cm.^{2}, representing 45 % of the total skin tattoo area of $2{\cdot}55 \pm 0{\cdot}14$ cm.^{2}.

There was no significant difference between the behaviour of the wounds of groups B and C in the 30 min. after the incisions were made, so the two groups can be pooled. The skin tattoo area, still held by the splint, did not change significantly during this time (mean expansion $0{\cdot}01 \pm 0{\cdot}07$ cm.^{2}). The isolated central granulations shrank sharply in area, losing $0{\cdot}69 \pm 0{\cdot}04$ cm.^{2} (approximately one-third of their initial area) at the same time rounding and bulging conspicuously above the skin surface. The central area, bordered by the outer edges of the incisions, in which the isolated papilla of granulations was situated, expanded by $0{\cdot}55 \pm 0{\cdot}06$ cm.^{2}, and this was balanced by a narrowing of the remaining peripheral rim of granulations and skin, which lost $0{\cdot}53 \pm 0{\cdot}08$ cm. in area.

The behaviour was different in group A, in which the wound had been allowed to contract down by removal of the splint 2 days before. The skin tattoo area expanded as soon as the incisions were made, gaining $0{\cdot}60 \pm 0{\cdot}09$ cm.^{2} in 30 min. This by no means returned the area to its size before the splint was removed; it was still only 56 % of this. The isolated central granulations shrank down as in the splinted groups, but not so conspicuously; they lost, however, about as much area proportionately to their size, diminishing by a mean amount of $0{\cdot}32 \pm 0{\cdot}06$ cm.^{2}. The central area bordered by the outer edges of the incisions, in which this isolated granulation tissue lay, expanded by $0{\cdot}65 \pm 0{\cdot}05$ cm.^{2}, while the peripheral rim, which so to speak loses area to the expanding central space and gains area from the expanding whole wound, did not change significantly in area (it shrank by $0{\cdot}06 \pm 0{\cdot}08$ cm.^{2}).

Later behaviour after excision and splint removal

After both excision of the greater part of the granulations and removal of the splints had been performed, a sample of the wounds was followed for a further 5 days. Wounds of groups B (6 specimens) and C (9 specimens) were excised and then desplinted at 10 and 12 days respectively; 30 min. after this was completed they had contracted down but did not differ significantly in mean skin tattoo area (group B $4{\cdot}11 \pm 0{\cdot}38$; group C $4{\cdot}27 \pm 0{\cdot}17$ cm.^{2}). During the subsequent 2 days they lost 1·28 ± 0·14 and 0·84 ± 0·13 cm.^{2} from these areas respectively, the difference between the groups being hardly significant (*t* 2·29, D.F. 13, *P* 0·05). By 5 days they had lost a total of 1·76 ± 0·07 and 1·83 ± 0·16 cm.^{2} respectively from their initial areas. Wounds of group A (6 specimens) were desplinted at 10 days and excised at 12 days. During the next 30 min. the wounds expanded, reaching a mean area of 3·10 ± 0·19 cm.^{2}. Their subsequent contraction was less than that of the other two groups, 0·64 ± 0·17 cm.^{2} in 2 days (which approximately nullified the effect of the immediate expansion on taking off the splint) and a total of 1·26 ± 0·10 cm.^{2} in 5 days.

During this period of 5 days the area of the hole from which the granulation tissue had been excised could be followed, although it quickly became occupied by new granulation tissue. This area started at 2·39 ± 0·14 cm.^{2} in groups B and C (which did not differ significantly) and 1·80 ± 0·14 cm.^{2} in group A. Significantly contractions had occurred in all groups at 2 days (the groups did not differ significantly; mean pooled loss 0·58 ± 0·06 cm.^{2}) and had gone further by 5 days (mean pooled loss 1·23 ± 0·08 cm.^{2}).

Chemical analysis

The piece of skin excised at the initial operation to prepare the wound was weighed and its nitrogen and hydroxyproline estimated. Tested by analysis of variance the excised pieces of the three groups (A, B and C) did not differ significantly in wet weight, dry weight, hydroxyproline content or total nitrogen content (in all cases the variance between groups was less than that within). Pooling all the specimens gave the mean weight of 1 cm.^{2} of normal skin as 125 ± 7 mg.; the mean percentage of water as 72·5 ± 0·8; the mean percentage of the dry weight attributable to collagen (the latter calculated from the hydroxyproline content by multiplying by 7·46) as 68·3 ± 2·1; and the mean amount of collagen in 1 cm.^{2} of skin as 23·7 ± 1·9 mg.

Analyses were also made of the excised pieces of granulation tissue. These pieces were as large as possible consonant with the avoidance of any possible contamination by the margin of original skin. They varied in absolute size in the three groups of wounds according to the amount of contraction that had occurred, but they were 43–45 % of the total skin tattoo area in all groups, and about two-thirds of the total area of granulation tissue. The absolute size was smallest in group A, which had been allowed to contract by removal of the splint at 10 days, biopsy being at 12 days. In groups B and C biopsy was at 10 and 12 days respectively, with the wound still splinted, and the sizes of these pieces were very similar to each other. The weights and chemical estimations are given in Table 3.

If these measurements are expressed per unit area of piece removed, the contracted wounds of group A have rather higher mean measurements in wet weight, dry weight, total N, hydroxyproline and DNA, than had the 12-day specimens of group C; though the difference is not nearly as much as would be expected if all that had happened to group A wounds was a shrinkage in area without change of content. The specimens of group C in turn had higher measurements than the 10 day specimens of group B (except in DNA, which was similar). When, however, the weight and chemical composition measurements were standardized for area by analysis of covariance, which is the appropriate statistical test, group A wounds differed in no instance significantly from the wounds of groups B or C. The wounds of group C significantly exceeded those of group B in wet weight, total N and hydroxyproline.

It is not possible to interpret the differences between groups B and C unequivocally, since it may be that the examination and measurement of the wounds at 10 days affected the wounds of group C (biopsied 2 days later) more than those of group B (biopsied almost immediately). The greater weight in group C, which is largely accounted for by a greater water content, may be thus explained. On the other hand, the increased collagen and total N in group C may represent a synthesis of intercellular material during these two days, such as might be expected from the estimation of collagen deposition in rats of Abercrombie *et al.* (1954), though not from those in guinea-pigs of Grillo *et al.* (1958).

Table 3. *Mean area, weight and composition, with standard errors, of samples of granulation tissue removed at biopsy*

All samples represent the same area of granulation tissue at 10 days. The wounds of group A were released from splinting and allowed to contract at 10 days, and were biopsied at 12 days. Those of groups B and C were biopsied while splinted at 10 and 12 days respectively. Each mean is of 10 specimens unless otherwise stated in brackets after the standard error.

Group	Area at biopsy (cm^2.)	Wet weight (mg.)	Dry weight (mg.)	Total nitrogen (mg.)	Hydroxy-proline (µg.)	DNA (µg.)
A	1·14 ± 0·10	267 ± 12	43·9 ± 2·0	6·3 ± 0·7	1660 ± 230	980 ± 160 (6)
B	2·28 ± 0·16	361 ± 32	65·8 ± 4·9 (7)	8·5 ± 0·6 (9)	1920 ± 150	1300 ± 130
C	2·03 ± 0·19	436 ± 40	65·6 ± 7·1	10·2 ± 1·2	2530 ± 240	1070 ± 170 (6)

The failure to find a significant difference in composition, when the measurements are standardized for area, between the contracted wounds and the splinted ones must be considered in relation to the fact that the contracted wounds had approximately half the total area of the others. It suggests that there had been a considerable loss of material during the two days of rapid contraction undergone by the wounds of group A, and this loss seems to have included collagen.

DISCUSSION

There are several possible mechanisms by which the edges of a skin wound might be moved inwards so as to diminish the size of the original defect. (1) They may be pushed in by extension of the surrounding skin. (2) They may by their own growth push themselves inwards, pushing against the surrounding skin. (3) There may be a sphincter-like action of contractile material at the wound edge. (4) The wound edges may pull themselves inwards, pulling on the material within the margins of the defect. (5) They may be pulled inwards by the material within the margins of the defect.

Both hypotheses (4) and (5) lead one to expect a detectable tension in the material within the wound; the other mechanisms, acting alone, would tend to compress this material. It is evident from the results of the present work that when wound contraction in rabbit skin is obstructed by glueing a splint to the surrounding skin, a considerable tension builds up in the granulation tissue filling the wound.

If, for instance, while still splinted, the central granulation tissue is isolated by incisions down to the panniculus it rapidly contracts into a button of tissue projecting conspicuously above the skin surface, while the rim of granulation tissue peripheral to the incisions retracts outwards against the edge of the hole in the splint. Lindquist (1946) has previously recorded the contraction of the isolated wound contents in splinted wounds of the rat.

That the isolated granulation tissue contracts does not establish that it has sufficient power to move the wound edge. Other data, however, make it probable that, in wounds that have previously been splinted, it actually drags the wound edges inwards. This is suggested by two pieces of evidence. (*a*) When the wound is released by removing the splint it shrinks rapidly in area; but if the central two-thirds of the area of granulation tissue has been excised just before removal of the splint, the reduction of area is much less rapid. (*b*) When, after removal of the splint, contraction has been allowed to occur (with granulation tissue intact) the surrounding skin is put under a counteracting tension, so that if the middle of the wound is then isolated by incisions the surrounding skin springs out again, while the middle contracts down a little further.

This evidence of an effective tension in granulation tissue concerns wounds which are or have been splinted. To what extent can our arguments be applied to unsplinted wounds? The contraction obtained is obviously quantitatively different from that occurring in unsplinted wounds. When the restraint of the splint is suddenly released the contraction is at first so fast as to be directly visible, and the wound area can be halved in two days. Nothing so spectacular happens with unsplinted wounds. There seem to be two hypotheses possible about the relation of this rapid to the normally slow contraction. (*a*) The rapid contraction represents an exaggeration of certain aspects of the normal process. It is evident, on this view, that contractile energy has been accumulated in the splinted wounds. It may be that contraction is normally slow because the members of a population of contractile elements each undergo a relatively brief main phase of contraction but at varying times; and that in the splinted wounds these individual contractile acts are synchronized when the constraint is released. It may also, or instead, be that elements in the wound are stretched by the contractile process, exaggeratedly so in a splinted wound. On release from splinting the elastic recoil of these elements may perform or contribute to the work of the sudden contraction. Whichever is correct, in so far as the surrounding skin resists the inward pull in the normal slow contraction, tension will develop in the granulation tissue. Evidence has in fact been presented for such tension in unsplinted wounds of the rabbit (Billingham & Medawar, 1955) and of the rat (Abercrombie *et al.* 1954; Cuthbertson, 1959), though Watts *et al.* (1958) did not find it in the guinea-pig. (*b*) The alternative hypothesis is that splinting or release therefrom sets going quite new contractile processes in granulation tissue. At present this alternative seems far-fetched, but it cannot be excluded.

Assuming that in splinted wounds we have been dealing with a quantitatively distorted version of normal wound contraction, we must next consider the mechanisms likely to be involved in engendering the tension in the granulation tissue. Watts *et al*, (1958) have suggested that wound contraction is produced by the activity of the wound edge, which migrates inwards. This is type (4) of the hypotheses listed at the beginning of the Discussion. Such a migration would produce a tension within the wound, since the moving cells would tend to pull their substrate in the opposite direction, a pull which would be exaggerated when the edge is substantially held in place by a splint. If the substrate of the moving cells is the granulation tissue, a possibility implied by the suggestion of Grillo *et al.* (1958) that the wound content becomes partly covered by the advancing skin edges, this should contract when isolated by incisions, as we have found. It is, however, impossible for the granulations in the splinted wound to be simultaneously pulled outwards by the skin edge and pushed inwards. Tattoo marks made on the granulations, we have shown, actually move inwards. The tension within the granulation tissue does not therefore result from its use as a substrate by the moving skin edge. Watts *et al.* (1958), however, suggest that it is the original wound base, not the granulation tissue, that the skin edge moves upon; in our experiments this base would be the panniculus (see Billingham & Russell, 1956), but in this case the granulation tissue would not be placed under tension. Hypotheses of type (4), such as that of Watts *et al.*, can be discarded as an explanation of the tension generated in the granulation tissue of splinted wounds of the rabbit, and therefore, as we have argued, of unsplinted wounds too.

Our data point clearly to a hypothesis of type (5), the 'granulous contraction' of Carrel (1910), which asserts that the tension is engendered by a process within the granulation tissue. The first mechanism to be considered is that a withdrawal of material from the granulation tissue is responsible.

Cuthbertson (1959) has suggested that (in the rat) contraction is related to decreased capillary permeability and dehydration of the wound bed. Any hypothesis of simple withdrawal of material from the granulation tissue seems to us inadequate to account for ordinary wound contraction, let alone the phenomena observed in contraction after splinting. Withdrawal would be expected to shrink the wound contents inwards from the surface relatively more than in superficial area, since resistance in the plane of the skin is greater. But the thickness of granulation tissue in an ordinary wound does not diminish with contraction, as Cuthbertson (1959) himself observed. When the splint is removed from a splinted wound the thickness of the granulation tissue actually *increases* while rapid contraction in the plane of the skin is going on, as Marshak (1945) described and as we have confirmed, which is all the more difficult for a withdrawal hypothesis. To make a hypothesis of withdrawal of substance account for existing data seems to require in principle a set of compartments in the granulation tissue from which material can be differentially removed in such a way as to stress elastic elements predominantly in the plane of the skin. Until a hypothesis that meets these rather complicated requirements has been proposed, the withdrawal of material cannot be taken seriously as an important cause of the tension in granulation tissue.

Hypotheses which postulate contractile elements, or contractile arrangements of elements, within the granulation tissue, predominantly parallel to the skin surface, have no difficulty in accounting for the tension in granulation tissue. It was for long rather uncritically accepted that this contractile component was the newly formed collagen. Abercrombie & James (1955), Abercrombie *et al.* (1956) and van den Brenk (1956) gave reasons for doubting whether collagen could fill this role, and they were followed in their doubts by Grillo *et al.* (1958). There are better grounds for thinking that the cells, or the arrangements of the cells, may provide the contractile force (Abercrombie *et al.* 1956) but direct evidence for this hypothesis is still lacking.

Acceptance of the view that the contraction of granulation tissue produces contraction of the wound does not mean of course that one or more of mechanisms (1)—(4) listed at the beginning of this Discussion do not play a subsidiary part. Some of our data are at least compatible with the operation of these other mechanisms, though it must be noted that they can with equal plausibility be explained without going beyond the hypothesis of contractile granulation tissue. Thus, if excision of the central two-thirds of the area of granulation tissue is done shortly before removal of the splint, then when the latter is removed the wound immediately contracts. This, however, is not unexpected if the rim of granulation tissue left behind is contractile like the centre, though it is possible that a mechanism causing inward pressure is concerned. If the excision is done 2 days after removal of the splint, the wound immediately expands, but not to its original size; and 2 days later it has contracted again. The failure to regain the original size is explicable perhaps by the rim of granulation tissue left behind, or by plastic deformation of the wound margin caused by the previous contraction, or even by growth of the wound margin under tension (Billingham & Medawar, 1955; James, 1959). The rapid resumption of contraction in spite of the excision (recorded also, in the guinea-pig, by Watts *et al.* 1958) may be due to the very quick formation of new contractile granulation tissue from a wound bed already activated to proliferation, or to redeployment of contractile elements in the remaining granulation tissue.

As Abercrombie *et al.* (1954) pointed out, a loss of substance from the granulation tissue accompanies normal contraction in the rat, though Grillo *et al.* (1958) did not find such a close relationship of wet weight and area in the guinea-pig. This loss of material is not likely to be an important cause of contraction (see above). It may be simply a result of changes in capillary permeability, as recorded by Cuthbertson (1959), running approximately parallel to the development of contraction in the granulations. It may, on the other hand, be the *result* of the contraction of the granulation tissue, which may raise tissue pressure. Our present data confirm that the loss occurs to a substantial extent in 2 days when contraction is sudden, though during the very fast initial phase of contraction after release from a splint it is evident that volume fails to diminish as area diminishes. There appears, however, to be more involved in the tissue loss than mere extrusion of diffusible material, since there is evidence that collagen is substantially reduced during the rapid contraction after release from splinting, so that collagen concentration does not rise significantly. Though we do not regard this evidence as yet entirely satisfactory, it is in accord with the finding of Grillo *et al.* (1958) that in the guinea-pig during normal contraction there is a phase when the wound is rapidly losing area and the collagen content falls. The apparent dissolution of the granulation tissue during contraction recalls the phrase of Billingham & Medawar (1955) that granulation tissue is ‘a temporary organ of repair’ and of Billingham & Russell (1956) that it is ‘an ephemeral organ of contracture’.

SUMMARY

1. About 4 cm.^{2} of skin was removed, down to the panniculus, from the sides of the chest in rabbits, and the resulting wounds were held by a splint glued to the surrounding skin. The wounds were dressed.

2. During the subsequent 10 days the wound as a whole achieved a slight contraction. The central part of the granulation tissue contracted more than the wound as a whole.

3. At 10 days after wounding. removal of the splint led to an immediate wound contraction, with at first some outward bulging of the granulations above the skin surface. Excision of the central area of the granulation tissue of such wounds just before splint removal reduced the subsequent contraction.

4. The central granulation tissue, when isolated by incisions in the splinted wounds, immediately contracted down, bulging above the skin surface. When similarly isolated in a contracted, de-splinted wound the central granulation tissue also contracted, while the whole wound expanded.

5. It is concluded that the granulation tissue is actively contractile, and contracts sufficiently powerfully to draw the skin edges inwards.

6. The dry weight, wet weight, total nitrogen, content of hydroxyproline and content of deoxyribose nucleic acid, when standardized to unit area, did not differ significantly between granulation tissue of de-splinted wounds which had contracted for 2 days, losing about half their area, and granulation tissue of wounds still held expanded by splints. This suggests a substantial loss of material, including collagen, during two days of rapid contraction.

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MORPHOLOGIC FEATURES OF THE HUMAN SUPRARENAL CORTEX IN MEN AGED 20–86 YEARS

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In spite of current interest in the physiology of the suprarenal cortex few serious attempts have been made to evaluate the morphology of the non-pathological human gland. This is possibly due in part to difficulty in obtaining material. Morphological data concerning suprarenal cortical zones in pregnancy (Whiteley & Stoner, 1957) and in summer and winter (MacKinnon & MacKinnon, 1958) have, however, been published.

Whiteley & Stoner (1957) measured the widths of four suprarenal cortical zones, the z. glomerulosa, the outer z. fasciculata, the inner z. fasciculata and the z. reticularis in twenty-five women who had died during pregnancy, and in forty-eight non-pregnant women who had died during reproductive life, and found that the inner z. fasciculata was significantly wider in pregnancy, no other significant changes having taken place in the remaining zones or in the total cortex. Histochemical appearances in the pregnant and non-pregnant glands were 'not strikingly different' although, on staining with Sudan IV, there appeared to be somewhat less sudanophilia in the pregnant ones.

MacKinnon & MacKinnon (1958) measured widths and also nuclear densities of the same four zones in women of reproductive age who had died suddenly in summer and winter. The inner z. fasciculata was significantly wider in summer, and nuclear densities in the z. glomerulosa and inner z. fasciculata greater in summer. No other zonal differences in width, nuclear density or sudanophilia were significant.

The aim of the present investigation was to add to the existing collection of morphological data concerning the four suprarenal cortical zones by measuring their width, nuclear density and sudanophilia in adult men of a wide age range.

MATERIALS AND METHODS

Right suprarenals from fifty-nine men, aged 20–86 years who died unexpectedly, were obtained from a series of autopsies carried out in a coroner's mortuary in London.

Bodies were refrigerated on arrival at the mortuary and the glands removed and placed in 10 % formal saline within 48 hr. of death. Following fixation the glands were cut into three parts at right angles to their long axes, a portion of one part being excised and reserved for lipoid staining. The remaining parts were embedded in paraffin, sectioned at $7{\cdot}5\,\mu$ and stained with haematoxylin and eosin.

Haematoxylin and eosin sections

Ten measurements of the width and nuclear density of four cortical zones, the z. glomerulosa, the outer z. fasciculata, and inner z. fasciculata and the z. reticularis were made from each gland, three from sections of the first and second parts and four from sections of the third. The criteria by which the zones were defined were that the z. glomerulosa lies under the capsule and consists of patchy nests of cells with deeply staining nuclei (Pl. 1, *G.*), the outer z. fasciculata consists of parallel columns of cells with pale vacuolated cytoplasm surrounded by narrow sinusoids (Pl. 1, *O.F.*), the inner z. fasciculata consists of less regular columns the cells of which are less vacuolated and more deeply stained (Pl. 1, *I.F.*), and the z. reticularis consists of an irregular pattern of groups of deeply stained cells (Pl. 1, *R.*).

Precautions observed in the choice of the parts of the sections from which the data were obtained were that they were always at right angles to the surface of the gland, that the columns of the outer z. fasciculata had an arcade arrangement at their outer ends and were continuous at their inner ends with inner fasciculate columns, that some medulla was visible as a guarantee of the presence of the entire reticularis, and that infoldings of the gland were avoided because it was observed that the cortex appeared to be distorted and to be thicker in the folds than elsewhere.

Widths of cortical zones were measured in each gland by means of an eyepiece micrometer which had been calibrated against a stage micrometer.

Nuclear densities in the four cortical zones were measured by counting the intact nuclei and the nuclear fragments in ten fields 0·042 mm.^{2}, that is in 0·00001323 mm.^{3}. The counts were then converted to the number in the nearest unit volume, that is, in 0·00001 mm.^{3}.

Sudan IV sections

The parts of the glands reserved for lipoid staining were embedded in gelatin, frozen, cut at 20μ and the sections stained with Sudan IV. One section from each gland was examined by low-power microscopy and the degree of colour in each of its four cortical zones estimated empirically; small amounts being represented by + and large amounts by + + +.

RESULTS

Table 1 lists the causes of death found at the necropsies, the age of the subjects, the mean widths of the suprarenal cortices and of their four zonal subdivisions.

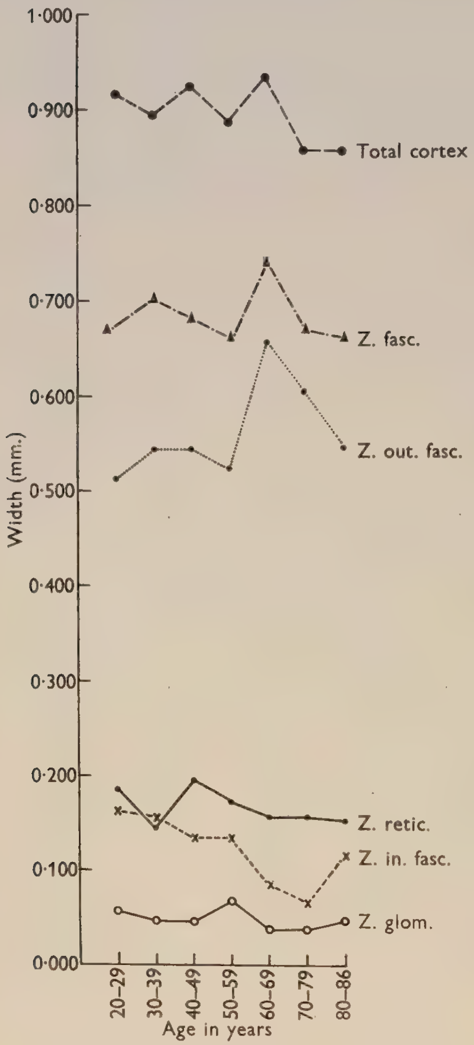
Graphs of the mean total cortical and zonal width per decade (Fig. 1) show that the total cortex was narrower in the 8th and 9th decades; the total z. fasciculata was wider in the 7th decade; the z. glomerulosa, the inner z. fasciculata and z. reticularis were narrower in the 7th to 9th decades; and the outer z. fasciculata was wider in the 7th to 9th decades. As widths of the outer z. fasciculata and inner z. fasciculata during the first three decades were in marked contrast with those of the last three decades, statistical analysis carried out by *t*-testing (Chambers, 1952), was confined to a comparison of figures for these two groups of decades in all zones. Significant differences were present in the widening of the outer z. fasciculata and z. reticularis. and in the narrowing of the inner z. fasciculata, in the later decades (Table 3).

Mean nuclear densities in the four cortical subdivisions of each gland are listed in Table 2. Their mean values per decade (Fig. 2) show that in each of the zones the density remained approximately constant during the 3rd to 5th decades and again, at a lower level, during the 7th to 9th decades. The difference between the means was significant in each case (Table 3).

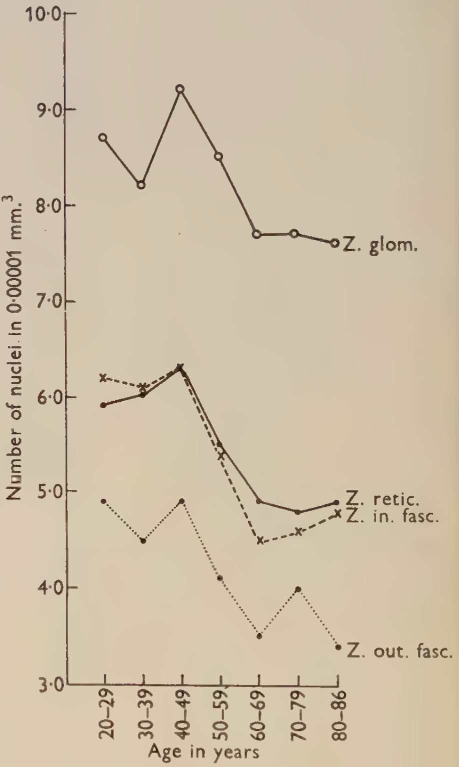
Table 1. *Widths* (mm.) *of total cortex and of constituent zones* (*each datum is the mean of ten observations*)

Male no.	Age	Cause of death	Glom.	Outer fasc.	Inner fasc.	Total fasc.	Retic.	Total cortex
1	20	Fract. skull	0·072	0·557	0·191	0·658	0·242	1·062
2	20	Fract. skull	0·053	0·520	0·142	0·662	0·099	0·814
3	24	Fract. skull	0·053	0·455	0·135	0·590	0·135	0·799
4	25	Carb. monox.	0·067	0·510	0·145	0·655	0·178	0·900
5	25	Carb. monox.	0·073	0·477	0·206	0·683	0·249	1·005
6	26	Carb. monox.	0·054	0·523	0·164	0·686	0·169	0·910
7	26	Carb. monox.	0·033	0·475	0·140	0·615	0·083	0·730
8	26	Fract. skull	0·074	0·621	0·202	0·823	0·175	1·072
9	28	Subarach. haem.	0·054	0·500	0·167	0·666	0·247	0·967
10	29	Coron. occl.	0·052	0·508	0·164	0·672	0·238	0·961
11	30	Fract. skull	0·045	0·530	0·186	0·716	0·187	0·948
12	34	Carb. monox.	0·036	0·618	0·116	0·734	0·144	0·914
13	37	Resp. failure	0·042	0·453	0·144	0·597	0·132	0·771
14	37	Coron. occl.	0·042	0·579	0·186	0·764	0·149	0·956
15	40	Carb. monox.	0·054	0·700	0·166	0·866	0·189	1·100
16	41	Carb. monox.	0·038	0·639	0·145	0·785	0·149	0·972
17	41	Coron, occl.	0·028	0·425	0·144	0·570	0·164	0·762
18	43	Coron. occl.	0·042	0·440	0·100	0·540	0·194	0·777
19	43	Coron. occl.	0·039	0·502	0·113	0·615	0·171	0·825
20	44	Hanging	0·039	0·576	0·128	0·704	0·254	0·996
21	45	Resp. failure	0·070	0·598	0·191	0·789	0·243	1·102
22	45	Coron. occl.	0·072	0·569	0·144	0·713	0·269	1·053
23	49	Coron. occl.	0·029	0·499	0·098	0·597	0·158	0·785
24	49	Coron. occl.	0·021	0·550	0·107	0·657	0·158	0·836
25	50	Carb. monox.	0·029	0·482	0·129	0·611	0·187	0·827
26	50	Coron. occl.	0·046	0·501	0·122	0·624	0·245	0·114
27	52	Coron. occl.	0·023	0·496	0·129	0·624	0·179	0·826
28	54	Carb. monox.	0·035	0·590	0·136	0·726	0·111	0·872
29	54	Barbiturates	0·053	0·563	0·081	0·644	0·136	0·834
30	56	Coron. occl.	0·046	0·531	0·115	0·629	0·142	0·816
31	57	Coron. occl.	0·072	0·525	0·180	0·706	0·204	0·982
32	57	Coron. occl.	0·042	0·508	0·154	0·662	0·156	0·861
33	58	Coron. occl.	0·076	0·607	0·155	0·762	0·250	1·089
34	59	Coron. occl.	0·045	0·494	0·135	0·629	0·160	0·834
35	61	Carb. monox.	0·039	0·647	0·075	0·721	0·144	0·904
36	62	Barbiturates	0·022	0·721	0·071	0·792	0·151	0·965
37	64	Coron. occl.	0·044	0·634	0·095	0·729	0·108	0·882
38	64	Coron. occl.	0·030	0·703	0·082	0·785	0·145	0·960
39	65	Coron. occl.	0·038	0·644	0.101	0·745	0·127	0·910
40	66	Coron. occl.	0·069	0·637	0·073	0·709	0·197	0·975
41	66	Pulm. embol.	0·061	0·635	0·046	0·681	0·148	0·890
42	67	Coron. occl.	0·027	0·758	0·106	0·864	0·135	1·026
43	67	Carb. monox.	0·023	0·601	0·121	0·722	0·207	0·952
44	67	Coron. occl.	0·035	0·618	0·098	0·716	0·180	0·931
45	71	Coron. occl.	0·032	0·602	0·074	0·675	0·186	0·876
46	72	Coron. occl.	0·008	0·552	0·060	0·612	0·128	0·749
47	75	Carb. monox.	0·035	0·621	0·063	0·684	0·124	0·843
48	75	Hanging	0·047	0·687	0·075	0·762	0·195	1·004
49	76	Coron. occl.	0·047	0·552	0·068	0·620	0·134	0·801
50	80	Coron. occl.	0·027	0·520	0·167	0·686	0·152	0·865
51	80	Coron. occl.	0·032	0·585	0·128	0·713	0·164	0·909
52	80	Fract. skull	0·060	0·561	0·080	0·641	0·191	0·892
53	80	Fract. skull	0·047	0·470	0·065	0·536	0·085	0·668
54	82	Coron. occl.	0·051	0·546	0·124	0·670	0·131	0·851
55	82	Coronl occl.	0·047	0·506	0·116	0·622	0·103	0·772
56	83	Thromb. aorta	0·064	0·596	0·143	0·739	0·179	0·982
57	84	Coron. occl.	0·026	0·554	0·079	0·633	0·100	0·759
58	85	Pulm. oed.	0·030	0·586	0·145	0·731	0·224	0·986
59	86	Coron. occl.	0·035	0·502	0·116	0·618	0·174	0·827

Empirical estimates of sudanophilia in the four cortical subdivisions of each gland are given in Table 2. Mean values per decade (Fig. 3) followed the same pattern in each zone, increasing at approximately 50 years of age, before and after which they remained approximately constant.



Text-fig. 1. Zonal width per decade.



Text-fig. 2. Zonal nuclear count per decade.

DISCUSSION

The methods by which the objectives of this investigation were reached contained certain fallibilities.

In order to obtain glands of a strictly physiological character they should, ideally, have been collected from men whose deaths were instantaneous and whose antecedent health was good. Obvious pathology previous to death was excluded at post mortem,

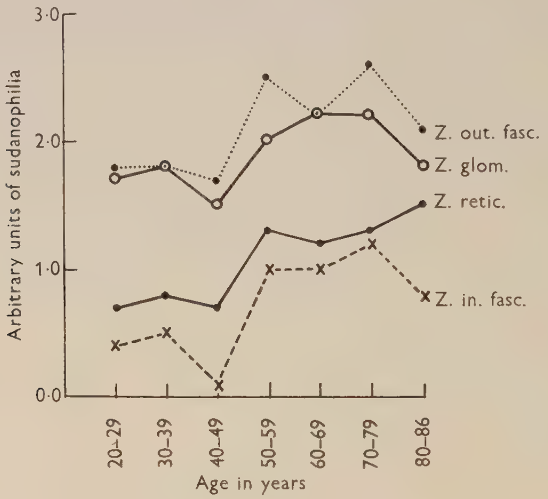
Table 2. *Number of nuclei in* 0·00001 mm.^{3} (*each datum is the mean of ten observations*), *and visual estimate of sudanophilia, in suprarenal cortical zones*

		Nuclear counts				Sudanophilia			
Male no.	Age	Glom.	Outer fasc.	Inner fasc.	Retic	Glom.	Outer fasc.	Inner fasc.	Retic.
1	20	7·5	4·7	5·3	4·8	+	+		
2	20	7·8	4·4	5·5	5·0	+	+		
3	24	10·5	7·2	8·2	7·7	++	++		
4	25	9·8	6·5	7·8	7·7	++	+++	+	+
5	25	7·5	2·9	6·0	4·2	++	++	+	+
6	26	9·4	5·5	7·1	6·4	+	+		++
7	26	9·8	5·1	6·6	8·2	++	++	+	+
8	26	7·8	3·9	4·6	4·0	+	+		
9	28	8·5	4·9	4·9	5·7	++	++		
10	29	8·7	4·1	5·7	4·8	+++	+++	+	++
11	30	8·5	4·1	5·4	5·8	++	++	+	++
12	34	8·5	5·1	6·4	6·2	+	+		
13	37	8·7	5·8	8·0	7·3	+	+		
14	37	7·1	3·0	4·5	4·6	+++	+++	+	+
15	40	7·8	3·9	4·6	4·6	+	++		+
16	41	8·6	5·0	5·7	5·8	+	++		+
17	41	10·7	6·1	7·1	5·7	++	++	+	+
18	43	8·7	5·1	5·2	5·8	++	++		++
19	43	9·7	5·5	8·1	7·6	++	++		
20	44	10·5	4·2	5·4	6·8	+	+		
21	45	8·7	4·6	7·2	6·9	++	+		
22	45	9·7	6·0	6·8	6·8	++	++		
23	49	9·0	5·1	6·6	6·0	++	++		
24	49	8·5	3·5	5·8	6·8	+	+		+
25	50	8·2	3·9	5·8	6·0	+++	+++	+	++
26	50	9·7	5·2	5·8	6·0	+	++		
27	52	11·3	5·2	8·0	7·6	++	++		
28	54	7·5	3·9	5·5	5·1	+++	+++	+	++
29	54	9·2	4·5	4·7	5·3	++	+++	++	++
30	56	8·0	3·4	4·4	4·3	+	++		
31	57	8·0	4·5	5·4	5·5	++	+++	++	++
32	57	8·3	3·9	6·0	5·8	++	++	+	++
33	58	8·6	2·5	3·9	4·9	++	++	+	+
34	59	6·1	3·4	4·3	4·6	++	+++	++	++
35	61	6·9	3·0	3·9	4·9	+	+		+
36	62	7·5	4·3	4·1	4·0	+++	+++	++	++
37	64	8·3	3·9	5·5	6·9	++	++	+	++
38	64	8·2	4·1	5·4	5·7	+++	+++	+	++
39	65	6·9	3·1	3·1	4·3	+++	+++	++	++
40	66	8·0	4·3	5·7	5·6	++	++		
41	66	8·2	3·1	3·7	4·1	++	++		
42	67	7·1	2·8	3·5	4·3	+++	+++		+
43	67	6·5	2·9	4·1	3·6	+	+		
44	67	8·0	3·6	5·6	5·8	++	++	++	++
45	71	8·2	4·7	3·6	3·5	++	++		
46	72	7·6	2·7	4·2	4·5	+	++	+	+
47	75	8·3	4·5	4·9	5·6	++	+++	+	++
48	75	7·5	4·4	4·9	6·4	+++	+++	++	++
49	76	6·9	3·9	4·7	4·1	+++	+++	++	++
50	80	7·2	2·8	4·1	3·9	++	++	+	++
51	80	6·5	2·9	3·6	4·2	+++	+++	++	++
52	80	5·8	2·8	4·1	5·0	++	++	+	++
53	80	7·5	3·8	5·0	6·0	++	+++	+	++
54	82	10·5	5·1	7·2	7·7	++	++		++
55	82	6·9	2·8	3·4	3·4	++	++	+	++
56	83	8·1	3·6	5·7	5·5	+	+		
57	84	7·7	3·5	5·3	4·1	++	+++	++	+++
58	85	8·3	3·6	5·7	5·4	+	++		+
59	86	7·2	2·7	3·4	4·2	+	+		

Table 3. *Mean width and nuclear density* (*no. nuclei in* 0·00001 mm.^{3} *of suprarenal cortical zones in men aged* 20–49 (*A*) *and* 60–86 (*B*) *years, with standard deviations*

(Probabilities were calculated by *t*-testing.)

		Width		Nuclear count	
Z. glomer.	(A) (B)	0·05 (±0·02) 0·04 (±0·02)	$P < 0.1$	8·8 (±2·1) 7·6 (±2·0)	$P < 0.001$
Z. out. fasc.	(A) (B)	0·53 (±0·12) 0·60 (±0·14)	$P < 0.05$	4·8 (±1·5) 3·6 (±0·9)	$P < 0.001$
Z. in. fasc.	(A) (B)	0·15 (±0·05) 0·09 (±0·01)	$P < 0.001$	6·2 (±1·8) 4·6 (±1·4)	$P < 0.001$
Z. total fasc.	(A) (B)	0·69 (±0·14) 0·70 (±0·16)	$P < 0.3$		
Z. retic.	(A) (B)	0·18 (±0·07) 0·15 (±0·05)	$P < 0.02$	6·1 (±1·8) 4·9 (±1·5)	$P < 0.001$
Total cortex	(A) (B)	0·92 (±0·22) 0·89 (±0·20)	$P < 0.6$		



Text-fig. 3. Sudanophilia per decade.

but the immediate causes of death varied considerably. The most common were coronary occlusion, carbon monoxide poisoning and fractured skull, the two latter being more frequent in the early decades. In the cases of carbon monoxide poisoning the time elapsed between the onset of unconsciousness and death was unknown, but the majority of the deaths from vascular accidents and fractures of the skull were known to have taken place within an hour of the incidents which caused them.

Coronary thrombosis is frequently associated with hypertension, a stress disease which gives rise to suprarenal cortical enlargement (Dawson, 1956), but there was no gross evidence of hypertensive changes at post mortem, nor evidence that the glands obtained from deaths due to coronary thrombosis were larger than the others (Table 1), nor was there any indication that sudanophilia was less in them (Table 2), as it should be in stress (Symington, Currie, Curran & Davidson, 1955).

The time which elapsed between death and autopsy is unlikely to have been a significant variable because it never exceeded 48 hr. and Zamchek (1947) found that suprarenals could be kept at room temperature for 56 hr. without affecting their histochemistry.

If, from the foregoing, a guarded assumption is made that the glands under review formed an acceptable sample of physiological suprarenals obtained from the right sides of adult males, the question then arises as to whether or not the measurements of cortical widths were realistic indices of the total suprarenal cortical tissue in each man. Swinyard (1940) stated, without reference to facts, that the left suprarenal was larger than the right, but Quinan & Berger (1933), who examined right and left glands in fifty 'selected coroner's cases', found that the mean weight of the right gland was 4·161 g. and that of the left 4·165 g. Causes of death were not given in every case but they were stated to have included gunshot wounds and deaths from car accidents, and it therefore seems probable that they were fully selected and that there is no significant difference in the normal size of the right and left suprarenal. Extrasuprarenal cortical tissue is present in 20 % of human subjects (Forsham & Thorn, 1955), but it was not sought for in the present series. In spite of this it would seem that the right gland is a reasonable representation of half the suprarenal cortical tissue in man. But, although this may be so, the linear measurements which were used as an assessment of total cortical tissue may not be strictly related to cortical volume, because no correlation of cortical width with cortical volume has, as yet, been published. Nevertheless, the suprarenal enlarges in a variety of diseases in man (Dawson, 1956; Sarazen, 1943) and if, as appears to be the case, the enlargement is largely confined to the cortex (Stirling & Keating, 1958), pathological enlargement is obviously reflected in cortical width, and it would be surprising if, within the physiological range, changes in volume were not also reflected in width.

MacKinnon & MacKinnon (1958) found that the mean summer–winter width of the suprarenal cortices of women, aged 18–46 years, who had died suddenly during the luteal phases of their menstrual cycles, was 0·915 mm. which approximates very closely to the width presently found in males aged 20–49 years. The similarity suggests that suprarenal cortical width is the same in the physiological state in males as in luteal females of corresponding age.

Other observers have found the suprarenal cortex to be wider. Stirling & Keating (1958), for example, found the mean width of the cortex in glands from twenty men and women of unspecified age to be 1·13 mm., and Goldzieher (1944) found it to be even wider. However, their glands were selected in such a way as to allow the introduction of considerable pathology.

The measurement of zonal nuclear density was a straightforward procedure into which no appreciable error could have been introduced.

From these considerations, it seems that the glands under review formed a reasonable sample of physiological male suprarenals and that their zonal widths and nuclear densities during the 3rd to 5th and 7th to 9th decades are applicable to men in these age groups.

With regard to the gerontological aspect of the investigation, the rather dramatic increase in sudanophilia in each zone from a constant low level during the 3rd to 5th decades to a constant high in the 7th to 9th decades, accompanied by a very significant decline in nuclear density from a constant high in the early decades to a constant low in the late ones, is evidence of the kind of atrophy which takes place in ageing (Korenchevsky, Paris & Benjamin, 1953).

Although the atrophy affected the four cortical zones equally when measured by a combination of changes in sudanophilia and nuclear density, a zonal differential appeared when it was measured by decline in nuclear population, that is, by the product of zonal width and zonal density, the reduction in the nuclear population of the inner z. fasciculata having been of the order of 75 %, and of the outer z. fasciculata 20 %, while the reductions in the glomerular and reticular zones were intermediate.

The decline in the nuclear population of the z. glomerulosa in old age suggests a reduction in glomerular activity. This implies a fall in aldosterone production because aldosterone synthesis is a known function of the z. glomerulosa (Ayres, Gould, Simpson & Tait, 1956). Such a view is contrary to the observation by Albeau-Fernet, Bugard & Romani (1958) that urinary aldosterone is unaffected by senescence; however, their observations involved eight subjects, only two of whom were free from vascular disease, and whose age range was limited to 53–74 years.

Albeau-Fernet, *et al.* (1958) estimated the urinary excretion of 17 ketosteroids in twenty-four men aged 50–85 years and found them to be reduced after the age of 65. If, as Landing (1955) suggests, androgenic ketosteroids are synthesized in the z. reticularis the fall in the nuclear population of the z. reticularis in old age may explain their reduction in the urine.

Solomon & Shock (1950) found that the ability of the suprarenal to secrete hormones of the 11–17-oxysteroid group, which are probably products of the z. fasciculata (Landing, 1955), 'is not grossly impaired in senescence', and Albeau-Fernet, *et al.* (1958) found that the ability of the cortex to react to ACTH, presumably via the fasciculate zone (Landing, 1955) was unimpaired in old age. These observations may be associated with the very substantial preservation of the nuclear population in the principal part, that is, in the outer part, of the z. fasciculata in old age.

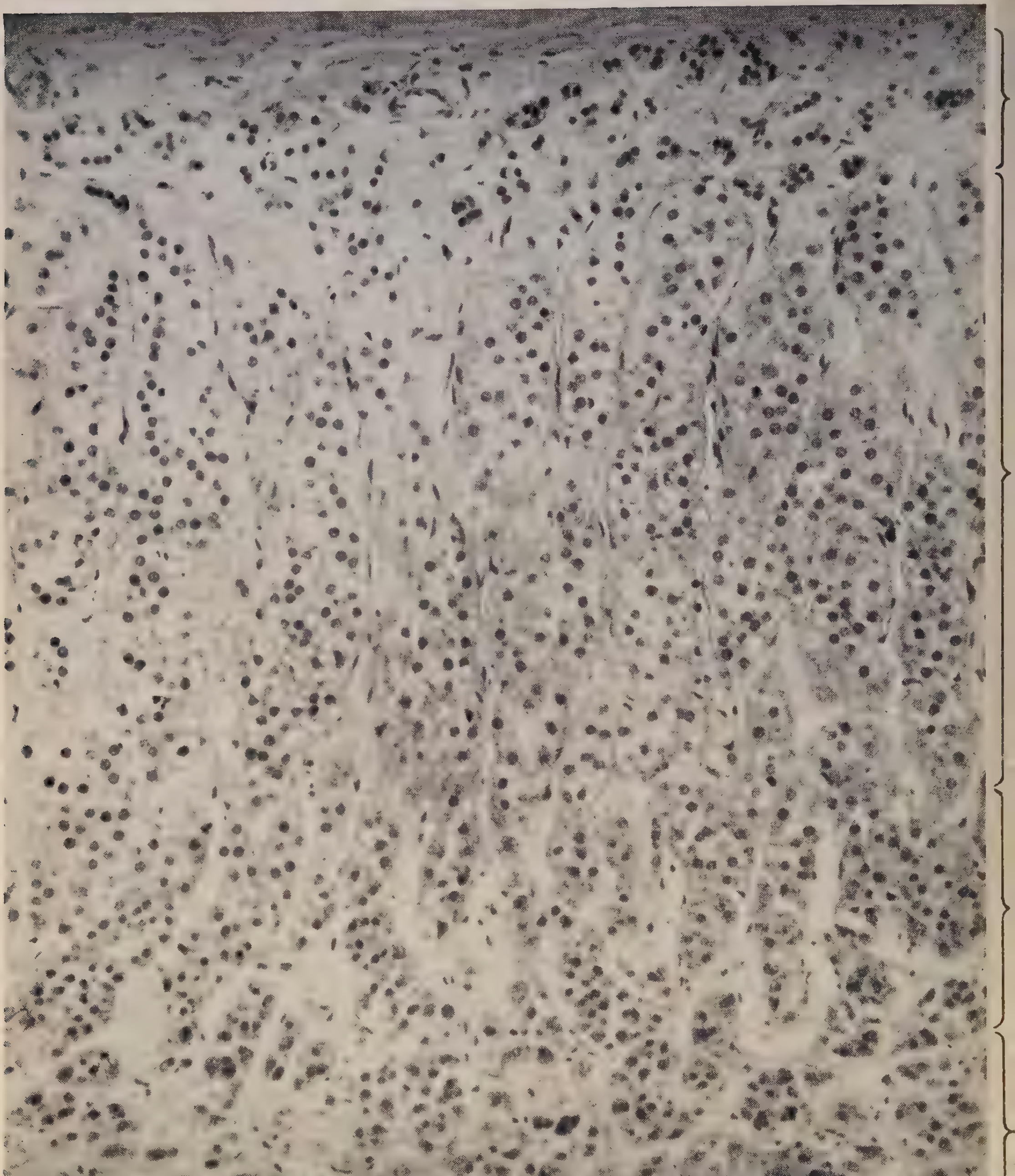
Information regarding the inner z. fasciculata in man is at present limited to the two morphological observations previously mentioned (Whiteley & Stoner, 1957; MacKinnon, & MacKinnon, 1958), and it would therefore be unwise to speculate seriously on the significance of the marked decline in the nuclear population of this zone in later life. But if it represents the same zone as was described by Chester Jones (1957) in experimental animals, and which was found by him to be influenced by gonadotrophic stimulation, then the marked changes which this zone undergoes in later life may well be associated with alterations in the sexual pattern.

SUMMARY

1. Width, nuclear density and sudanophilia were measured in four suprarenal cortical zones, the z. glomerulosa, the outer z. fasciculata, the inner z. fasciculata and the z. reticularis of right suprarenal glands of fifty-nine men who died suddenly, aged 20–86 years.

2. Signs of ageing atrophy were apparent in and around the 6th decade, therefore measurements of zonal width and nuclear density during 3rd–5th decades were compared statistically with those of 7th–9th decades.

3. In the later decades zonal width was significantly narrower in the inner z. fasciculata but significantly wider in the outer z. fasciculata and z. reticularis; nuclear



MacKINNON and MacKINNON—Morphology of suprarenal cortex density was significantly less in all zones; and sudanophilia was increased in all zones.

4. Assessment of nuclear population (zonal width × nuclear density) showed that ageing atrophy was greatest in the inner z. fasciculata and least in the outer z. fasciculata.

We wish to extend our thanks to Dr F. Camps who made this investigation possible.

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EXPLANATION OF PLATE

Photomicrograph of a 7·5 μ thick injection of human suprarenal cortex, stained with haematoxylin and eosin.

G = zona glomerulosa; O.F. = outer zona fasciculata;
I.F. = inner zona fasciculata; R = zona reticularis. ×250.

THE EYE AT BIRTH: MEASUREMENT OF THE PRINCIPAL DIAMETERS IN FORTY-EIGHT CADAVERS

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Though earlier measurements are extant, the first reliable value for the sagittal diameter of the eye at birth was that recorded by Thomas Nunneley in 1858. The average of the four eyes of two newborn infants is given as $\frac{22}{30}$ in., corresponding to 18·7 mm. Three years later von Jaeger gave the average for 70 eyes of 'new-born' infants as 17·53 mm. It is not clear whether in either case the term 'newborn' was used in its strict sense. In a series of 26 eyes, Merkel & Orr in 1892 gave the sagittal diameter as 17·5 mm., and the same value is recorded by Schneller in 1898 for 32 eyes. Various other averages are given by different observers for relatively small series, but that of Weiss (1897), which recorded a value of 16·4 mm. for 14 eyes of infants aged 1 to 5 days, is widely accepted. A value of 17·6 mm. is computed by Scammon & Armstrong (1950) on an empirical formula developed by Scammon (1925) for the entire foetal period. The literature does not always distinguish between full-term and premature babies, and the actual age is generally in doubt. It would seem that the average sagittal diameter at birth for the full-term baby is not less than 16 and not more than 18 mm.; it is probably around 17·5 mm.—a value higher than generally given in textbooks.

Data on the transverse and vertical diameters of the globe are even more scanty. For the transverse diameter there are the observations by von Jaeger (50 eyes), Weiss (14 eyes) and Schneller (33 eyes), and the respective averages are 17·2, 16·4 and 17·0 mm. For the vertical diameter the only relevant data are those by Weiss, who recorded an average of 15·0 mm. Scammon & Armstrong give computed values of 17·1 mm. for the transverse and 16·5 for the vertical diameter.

There are also some desultory data on the diameters of the cornea. Computed values of 9·8 mm. for the horizontal diameter and 9·0 mm. for the vertical diameter are given by Scammon & Armstrong, whilst Schneller gives 9·9 and 9·2 mm. for the two diameters respectively as the average of 33 measurements in cadavers. In the living subject Kaiser (1925) records 9·44 mm. for the horizontal diameter in 55 observations on infants aged up to 14 days, and Hymes (1929) gives 10·0 ± 0·2 mm. for 11 eyes of male infants and 9·9 ± 0·1 mm. for six eyes of female infants aged up to 10 days. This paucity of observations is rather unexpected, as the measurement of the corneal diameter presents relatively little difficulty.

In the course of the recent Perinatal Mortality Survey under the auspices of the National Birthday Trust Fund, Dr L. Crome collected the eyes of 48 cadavers of newborn infants, and kindly passed them on to us. The eyes had been removed

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* Working with a grant from the Alexander Piggott Wernher Memorial Trust, Medical Research Council.

generally within 24 hr. of death, and only those not collapsed were used for this study. They had been put in 5 % formol saline, and measurements of the globe and cornea were carried out within a few hours to a week after fixation, and repeated 6 weeks later. Both series of measurements agreed closely. The values recorded were the three diameters of the globe and the vertical and transverse diameters of the cornea; they are set out in Appendix A. It will be seen that in all there were thirty-four infants who had died within 24 hr. of birth; of the fourteen who lived for more than 24 hr., only one had lived as long as 12 days. Both eyes were measured in all but six infants, giving a total of 90 eyes.

ANALYSIS OF RESULTS

Sagittal diameter of the globe

Table 1 summarizes the data obtained. It will be seen that the sagittal diameter in full-term infants who lived less than 24 hr. was 17·9 ± 0·49 mm. for thirteen boys and 17·7 ± 0·42 mm. for six girls. For 7 full-term infants who lived more than 24 hr. (up to 6 days) the values were very much the same. In premature infants, the axial length follows fairly closely the birth weight. As can be seen from the data in the Appendix, in six infants with a birth weight or a history suggestive of prematurity the axial length once again follows birth weight.

Table 1. *Diameters of the globe and of the cornea (in mm.) in full-term and premature babies up to 6 days old*

			Globe			Cornea	
	No. of subjects by sex	No. of eyes	Sagittal, mean and S.D.	Vertical, mean and S.D.	Transverse, mean and S.D.	Transverse, mean and S.D.	Vertical, mean and S.D.
			Full Term				
less 24 hr.	M. 13	24	17·9 ± 0·49	17·3 ± 0·53	18·4 ± 0·608	9·8 ± 0·33	10·4 ± 0·35
	F. 6	12	17·7 ± 0·42	17·3 ± 0·45	18·1 ± 0·42	10·1 ± 0·33	10·7 ± 0·29
1–6	M. 3	6	17·7 ± 0·17	16·8 ± 0·13	17·7 ± 0·12	9·25 ± 0·404	10·1 ± 0·51
	F. 4	8	17·8 ± 0·36	17·2 ± 0·47	18·2 ± 0·44	10·1 ± 0·301	10·6 ± 0·301
			Prematures				
less 24 hr.	M. 6*	11	14·3 ± 1·4	13·4 ± 1·1	14·2 ± 1·05	7·5 ± 0·809	8·0 ± 0·88
	F. 3†	5	14·3 ± 1·4	13·4 ± 1·2	14·3 ± 1·3	7·9 ± 0·63	8·3 ± 0·63
1–6	M. 4‡	7	15·5 ± 1·08	14·7 ± 1·4	15·5 ± 1·3	8·5 ± 0·69	9·0 ± 0·88
	F. 2§	4	15·6 ± 0·69	14·8 ± 0·52	15·4 ± 0·82	8·9 ± 0·45	9·2 ± 0·16

* Birth weight: 1 lb. 12 oz.–3 lb. 11 oz.
† Birth weight: 1 lb. 3 oz.–3 lb. 0 oz.
‡ Birth weight: 2 lb. 0 oz.–4 lb. 11 oz.
§ Birth weight: 2 lb. 15 oz.–4 lb. 0 oz.

Vertical and transverse diameters of the globe

The data in Table 1 suggest that in full-term babies the vertical diameter at birth is shorter and the transverse longer than the axial length. In prematures the same relationship obtains: the axial length and transverse diameter are very much the same, but the vertical diameter is definitely the shortest.

Diameters of the cornea

In both full-term and premature babies, the values recorded for girls were higher than for boys. The transverse diameter was consistently larger than the vertical diameter.

DISCUSSION

The validity of measurements of the globe in post-mortem material is open to question. Until a suitable series is available which gives values on eyes measured immediately after death, and remeasured after being kept in fixatives, it is impossible to give a final answer. The observations recorded in the present study suggest that there is at any rate no substantial shrinking or swelling when eyes are kept in fixatives for some weeks.

The evidence on the validity of measurements of the cornea is rather more definite. Axenfeld (1897) measured the radius of curvature of corneae of adults both before death and within a few hours after death and found no change. The close agreement in the values for horizontal diameters of the cornea as measured in life by Kaiser and by Hymes, in the cadaver by Schneller, and between both these and the computed value given by Scammon & Armstrong, suggests that the measurements obtained post-mortem in an eye that is not collapsed can be taken as reasonably accurate. Accepting the values for the newborn eyes obtained in the present series, it is clear that the growth of the cornea follows a different pattern from that of the globe as a whole. The adult value for the transverse diameter of the cornea is generally given as between 11·0 and 11·8 mm. (Hymes, 1929). There is therefore an increase in diameter of some 2·0 mm. In contrast, the axial length increases during growth by some 6 mm. The increase in the cornea is therefore of the order of some 20 % and that of the axial length of some 33 %. In the eye in particular, size is only one aspect of growth; changes in the curvatures of the refracting media are of considerable significance. Axial elongation by some 6 mm. would represent a change in refraction of some 25 D., unless counteracted by flattening of the curvatures of the cornea and lens. There are now ample data on the changes in curvature after the age of 3 years (Sorsby, Benjamin & Sheridan, 1960); data on the eye at birth and in the first 3 years of life have still to be obtained.

SUMMARY

1. The scanty literature suggests that at birth the sagittal diameter of the eye is not less than 16 mm., and not more than 18 mm., and that the transverse diameter is of the same order and is perhaps longer than the vertical diameter. The transverse diameter of the cornea is given as 9·0–10·0 mm.

2. In a study on forty-eight cadavers of infants, forty-one fell into easily defined categories. The sagittal diameter of the globe in thirteen full-term boys who had lived for less than 24 hr. was found to be 17·9 ± 0·49 mm.; the value for six girls was 17·7 ± 0·42 mm. Similar values were found in seven full-term infants living up to 6 days. In fifteen premature infants the size of the globe was proportionate to birth weight: measurements of 12·1 to 16·8 mm. were recorded for the sagittal diameter. In both full-term and premature infants the vertical diameter was the shortest.

The transverse and vertical diameters of the cornea for full-term boys who lived less than 24 hr. were 9·8 ± 0·33 mm. and 10·4 ± 0·35 mm.; for girls they were 10·1 ± 0·33 and 10·7 ± 0·29. Lower readings were obtained for prematures, but here too the girls showed the larger values.

3. Comparing the values for the diameters of the globe and of the cornea in the newborn with those seen in the adult, it is clear that the scleral coat grows more than the cornea in size. In refracting surfaces, such as the cornea and lens, changes in curvature are however more significant than dimensional growth.

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APPENDIX A

Diameters of the globe and of the cornea of the eyes of forty-eight cadavers of newborn infants

Body weight at birth (lb. oz.)	Eye	Axial	Diameters (mm.)				Remarks
			Globe		Cornea		
			Vertical	Transverse	Vertical	Transverse	
				(1) Males			
Lived less than 24 hr.							
1 12	R.	12·9	12·2	13·0	6·5	7·1	3 months premature
1 14	R.	13·0	12·6	13·2	7·0	7·4	3 months premature
	L.	12·9	12·6	13·1	7·0	7·4	
2 5	R.	13·5	12·7	14·0	6·9	7·3	3 months premature
	L.	13·6	12·3	13·7	7·1	7·6	
2 8	R.	13·9	13·5	13·9	7·5	7·7	3 months premature
	L.	14·2	13·3	14·0	7·5	7·7	
3 1	R.	14·8	13·9	14·7	7·5	8·1	2½ months premature
	L.	14·8	13·7	14·9	7·5	8·0	
3 11	R.	16·8	15·2	16·1	9·1	9·6	2 months premature
	L.	16·5	15·5	15·8	8·9	9·7	
5 0*	R.	16·8	16·3	17·2	9·1	9·5	1 month premature
	L.	16·8	16·4	17·4	9·0	9·6	
5 2*	R.	16·2	15·6	16·2	9·4	9·6	Anomaly in lungs
	L.	15·9	15·7	16·3	9·6	9·8	
6 5	R.	17·6	16·6	17·8	9·5	9·9	Asphyxia
	L.	17·4	16·5	17·6	9·6	9·9	
7 0	R.	17·9	17·3	18·7	10·0	10·5	Asphyxia. Cord around neck
	L.	17·6	17·1	18·6	9·8	10·2	
7 2	R.	17·5	17·0	17·7	9·8	10·7	Asphyxia
7 2	R.	17·8	17·5	18·3	10·0	10·6	Asphyxia. Haemothorax
	L.	17·7	17·5	18·3	10·0	10·5	
7 2	R.	18·2	17·4	18·4	9·8	10·2	Subdural haemorrhage
	L.	18·4	17·7	18·5	9·9	10·2	
7 14	R.	18·3	17·8	18·9	9·7	10·7	Haemorrhage
	L.	18·2	17·9	19·2	9·8	10·6	
8 2	R.	18·0	16·9	18·0	10·2	10·7	Heart stopped before delivery
	L.	18·0	17·0	18·1	10·2	10·7	
8 2	R.	18·6	17·6	18·5	9·2	10·7	Anoxia (from uterine inertia)
8 10	R.	17·3	16·8	17·5	9·4	10·0	Anoxia
	L.	17·2	16·8	17·5	9·4	9·9	
9 1	R.	17·3	16·8	17·5	9·4	10·0	Subdural haemorrhage
	L.	17·6	16·6	17·6	9·4	9·9	
9 8	R.	18·0	17·3	18·6	9·4	10·2	Anoxia. Liquor in lungs
	L.	17·9	17·3	18·6	9·5	10·2	
9 15	R.	18·2	18·0	19·3	10·1	10·6	Subdural haemorrhage
	L.	18·3	17·9	19·2	10·0	10·5	
10 0	R.	18·8	18·2	19·1	10·2	10·8	Subdural haemorrhage
	L.	19·1	18·3	19·2	10·4	11·1	
Lived more than 24 hr.							
2 0	R.	13·5	12·4	13·0	7·2	7·5	Lived 25 hr. 3 months premature
3 0	R.	15·2	14·0	15·3	8·5	8·8	Lived 36 hr. 5 weeks premature
	L.	15·2	14·0	15·1	8·2	8·7	

* Uncertain whether premature or full term; excluded from Table I.

APPENDIX A (*cont.*)

Body weight at birth (lb. oz.)	Eye	Axial	Diameters (mm.)				Remarks
			Globe		Cornea		
			Vertical	Transverse	Vertical	Transverse	
Lived more than 24 hr. (*cont.*)							
3 8	R.	15·5	14·9	15·8	8·5	9·2	Lived 23 days. 5 weeks premature
	L.	15·5	15·1	15·8	8·6	9·0	
4 11	R.	16·7	16·4	16·7	9·4	10·1	Lived 46 hr. 3 weeks premature
	L.	16·7	16·0	16·5	9·0	10·0	
4 12	R.	17·7	16·9	17·7	9·5	10·2	Lived 51 hr. Term
	L.	17·8	16·9	17·6	9·6	10·3	
5 1	R.	17·5	16·8	17·6	8·9	9·4	Lived 26 hr.
	L.	17·5	16·9	17·7	8·6	9·5	
6 4	R.	17·9	16·6	17·9	9·4	10·6	Lived 6 days
	L.	17·8	16·7	17·8	9·5	10·5	
6 8	R.	17·8	17·2	18·4	10·2	10·8	Lived 12 days
	L.	17·9	17·3	18·4	9·9	10·5	
				(2) Females			
Lived less than 24 hr.							
1 3	R.	12·1	11·5	12·3	6·9	7·4	3 months premature
2 14	R.	14·2	13·4	14·2	7·9	8·2	3 weeks premature
	L.	14·3	13·4	14·4	7·9	8·2	
3 10	R.	15·5	14·4	15·3	8·4	8·8	2 months premature
	L.	15·5	14·4	15·5	8·5	9·0	
4 12*	R.	16·4	16·0	16·3	9·5	10·5	1 month premature
	L.	16·6	15·9	16·3	9·5	10·5	
5 1½*	R.	17·1	15·9	16·5	9·2	9·6	2 weeks premature
	L.	17·1	16·2	16·5	9·3	9·8	
5 14*	R.	17·4	16·0	16·5	9·8	9·9	3 weeks premature
	L.	17·5	16·5	17·5	9·8	10·0	
5 15*	L.	16·1	15·2	15·8	9·3	10·1	3 weeks premature
5 8	R.	17·6	17·0	18·0	10·2	10·9	Died of pneumothorax
	L.	17·5	16·9	18·0	10·5	10·8	
6 4	R.	17·3	16·9	17·5	10·2	10·7	Achondroplasia; foetal distress
	L.	17·3	17·0	17·6	10·2	10·6	
6 13	R.	18·0	17·5	18·3	10·3	10·8	Still-birth; ruptured foetal vessels in placenta
	L.	17·8	17·6	18·3	10·0	10·6	
7 1	R.	17·2	16·7	17·7	9·4	10·1	Hydrocephalus spina bifida
	L.	17·2	16·8	17·9	9·7	10·2	
7 10	R.	18·4	18·0	18·8	10·3	10·9	Asphyxia (aspiration of meconin)
	L.	18·4	18·0	18·8	10·4	11·0	
8 9	R.	17·5	17·2	17·9	9·7	10·9	Asphyxia (aspiration of meconin)
	L.	17·7	17·5	18·1	10·0	10·9	
Lived more than 24 hr.							
2 15	R.	15·0	14·4	14·6	8·9	9·2	1 month premature. Lived 24 hr.
	L.	15·0	14·3	14·8	8·7	9·4	
4 0	R.	16·2	15·3	16·2	9·0	9·2	1 month premature. Lived 2 days
	L.	16·2	15·2	16·0	9·0	9·0	
5 11	R.	18·1	17·5	18·6	10·4	10·8	Lived 6 days
	L.	18·1	17·4	18·5	10·5	11·1	
7 6	R.	17·3	16·5	17·5	10·2	10·6	Lived 3 days
	L.	17·2	16·5	17·5	9·5	10·1	
7 8	R.	18·1	17·7	18·5	10·1	10·8	Lived 6 days
	L.	17·9	17·5	18·3	10·0	10·7	
7 10	R.	17·9	17·3	18·4	10·2	10·4	Lived 4 days
	L.	17·9	17·5	18·3	10·1	10·5	

* Uncertain whether premature or full term; excluded from Table 1.

THE ADHERENCE BETWEEN THE FREE END OF THE BOVINE PENIS AND ITS SHEATH

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INTRODUCTION

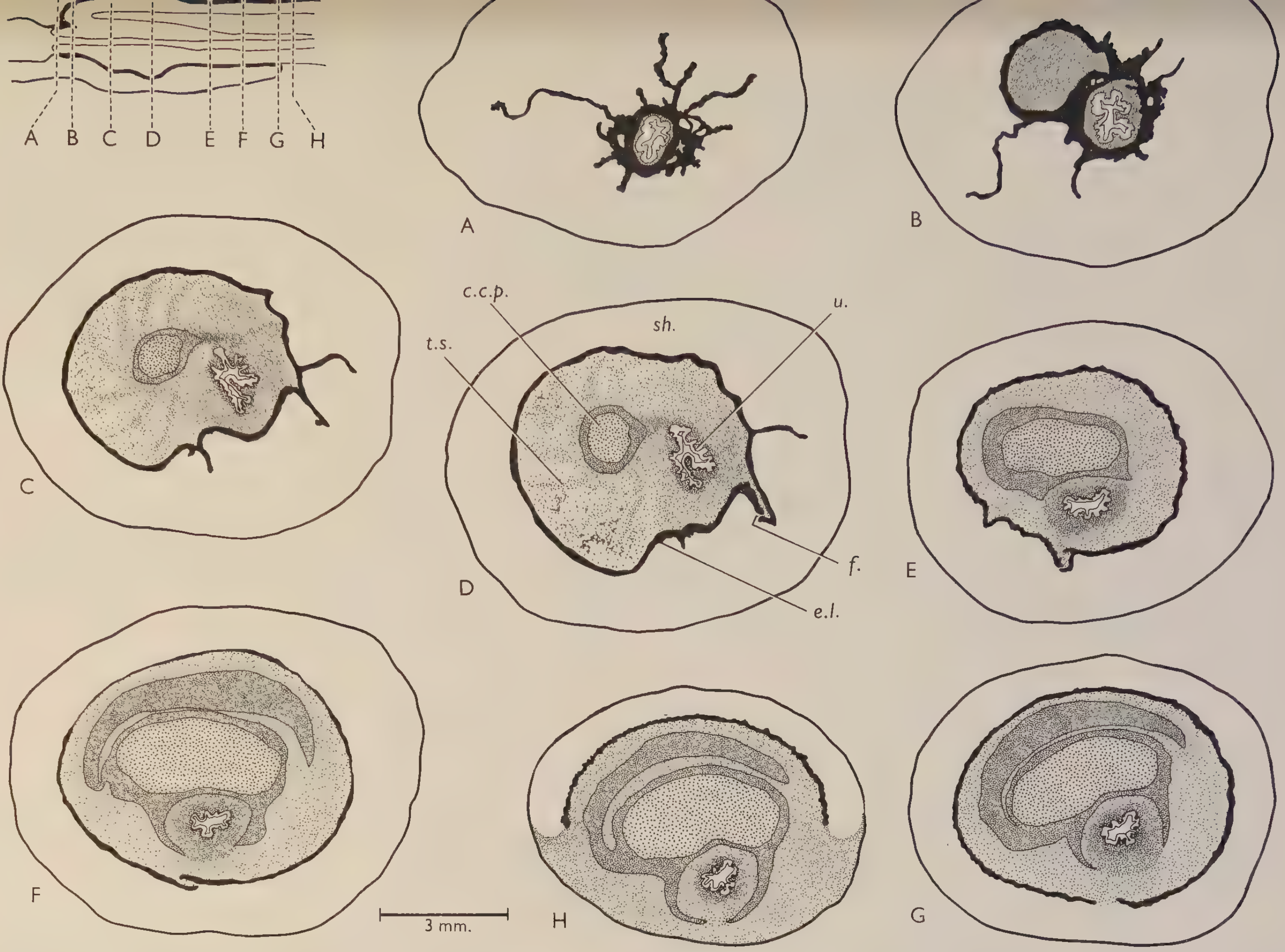
The free end of the penis of the ox is firmly adherent to its sheath at the time of birth (Colucci, 1872). The embryological development of the penis in the domesticated ruminants has been described in detail by Böhm (1905) and Broman (1947), but they were principally concerned with the early stages during which the penis is formed from the genital tubercle and the primitive *penis pendulus* is transformed into the definitive *penis appositus*. Little has been written about the later changes that take place in the penis and sheath. As a basis for further studies on the postnatal development of these organs in the bull I have therefore investigated the anatomical relationship between penis and sheath in young calves and foetuses.

MATERIALS AND METHODS

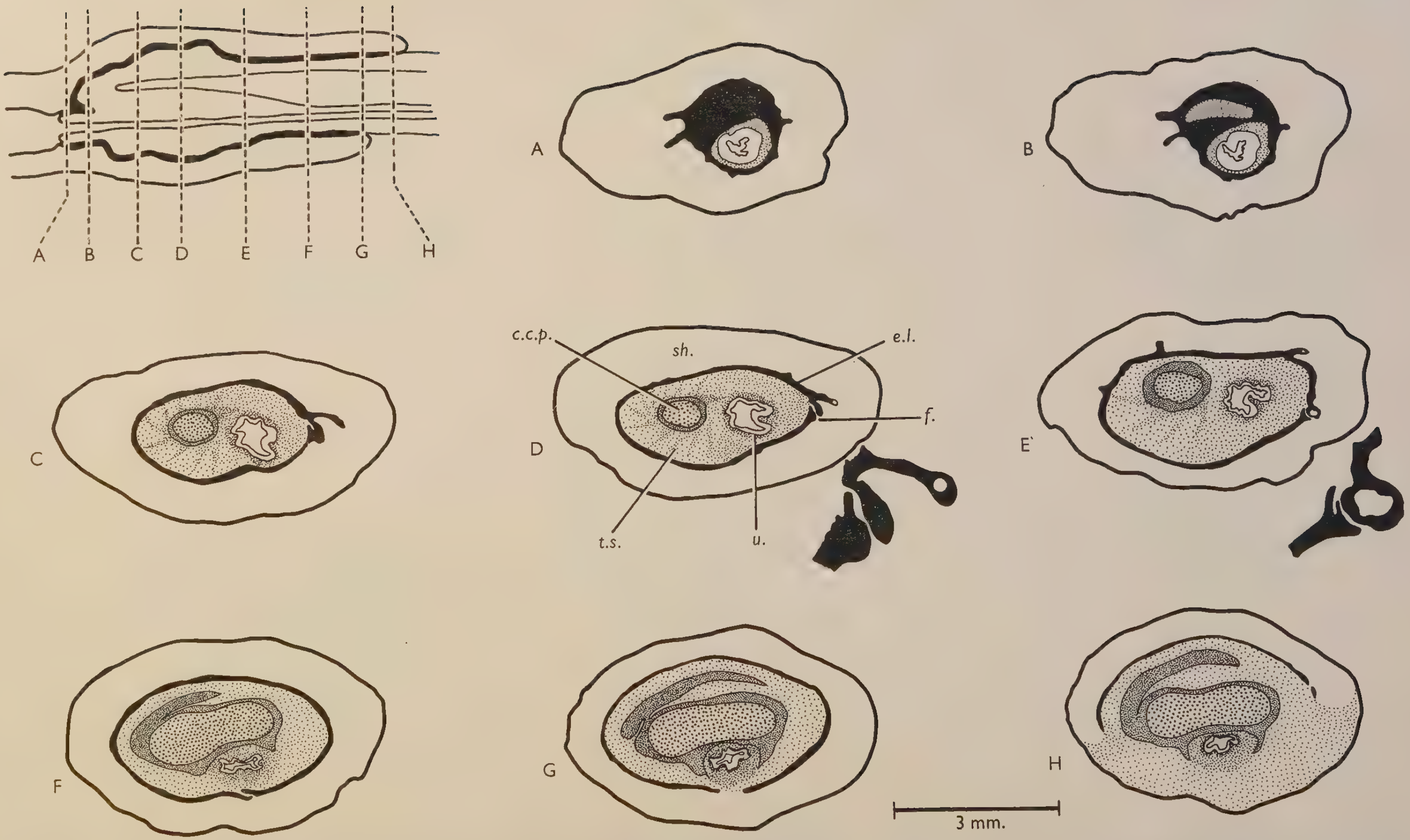
Histological material consisting of the whole of the free end of the penis with its adherent sheath was taken from five calves aged between 1 and 3 weeks and ten foetuses (135 mm., 23, 26, 36, 37, 43·5, 49, 58, 70, 73 cm. C.R. length). The specimens were fixed in formal saline, Bouin's fluid or Heidenhain's Susa; embedded for transverse sectioning in paraffin wax; serially sectioned at thicknesses of 10, 15, or 20μ; and mounted at intervals of 100μ. In the three smallest foetuses and one calf all sections were mounted. Sections were stained with haematoxylin and eosin, or haematoxylin and van Gieson's stain.

RESULTS

The penis of the ox lies beneath the skin of the ventral body wall and its apex is enclosed by the penile portion of the sheath. The orifice of the sheath lies just caudal to the umbilicus. The lumen of the pre-penile portion of the sheath is patent throughout foetal life, but the wall of the penile portion is adherent to the free end of the penis during foetal and early postnatal life. Two distinct tissues are involved in this adherence. Over the surface of the penis a thin lamella of ectodermal cells is adherent to the penis and to the sheath. This is the 'glandar lamella' of Böhm and the 'glando-preputial invagination' of Retterer. Near the urethra the ectodermal lamella is incomplete; its edges overlap without joining and a frenulum of connective tissue joins the penis to the sheath. Text-fig. 1 shows the arrangement of these tissues in the young calf and Text-fig. 2 shows this for a foetus of 23 cm. C.R. length. In the following account the tissues are described in the calf and then compared with the foetal material.



Text-fig. 1. Camera lucida drawings of transverse sections through the penis and sheath of a young calf to show the anatomy of the ectodermal lamella and the frenulum. (The diagram in the top left-hand corner shows the approximate levels of sections A–H along the free end of the penis and is not to scale.) A, through the urethral process, near the urethral orifice; B, through the urethral process, near its base, showing the apex of the terminal swelling ('galea-glandis'); C, D, through the terminal swelling; E–G, through the stalk of the free end ('collum glandis'); H, through the base of the free end, where there is a dorsal diverticulum of the sheath. *c.c.p.*, corpus cavernosum penis; *e.l.*, ectodermal lamella; *f.*, frenulum; *sh.*, penile sheath; *t.s.*, terminal swelling; *u.*, urethra, surrounded by the corpus cavernosum urethrae.



Text-fig. 2. Camera lucida drawings of transverse sections through the penis and sheath of a calf foetus (c.r. length 23 cm.) to show the anatomy of the ectodermal lamella and the frenulum. (The diagram in the top left-hand corner shows the approximate levels of sections A–H along the free end of the penis and is not to scale.) The levels are similar to those in Text-fig. 1 except that section E passes through the base of the terminal swelling. Insets to sections D and E show detail of the frenulum. Abbreviations as in Text-fig. 1.

(1) *The ectodermal lamella*

In these specimens the lamella was not intact at the urethral orifice; a rim 50–100 μ in height was more or less free from the sheath and small cavities extended into the lamella alongside the urethral process to a depth of 1 mm. Immediately behind the urethral orifice the lamella completely encloses the apical one-sixth of the penis and in transverse section it is a solid, uninterrupted ring of tissue (Text-fig. 1 A–C). However, over the remaining length of the free end the ventral borders meet or overlap leaving a frenulum of varying shape and size between them. Over most of the free end the lamella is an almost complete circle in transverse section (Text-fig. 1 D–G), but at the base of the free end it is semicircular (Text-fig. 1 H).

Over-all, the lamella is 130–150 μ thick: at the apex of the penis it is irregular in cross-sectional shape and its thickness may exceed 500 μ, while at the base of the free end, in the dorsal diverticulum, it decreases to 80–100 μ. The ventral sector of the lamella is somewhat thicker than the dorsal sector and is often corrugated.

The histological structure of the ectodermal lamella is shown in Pl. 1, figs. 1–4. It comprises from 5 to 40 layers of cells; over most of its extent it is 10–12 cells thick. It can be divided into three distinct zones (Pl. 1, fig. 3).

The outer zone (*stratum germinativum* of the penile sheath) comprises 1–3 layers of columnar cells resting upon an eosinophilic basement membrane.

The inner zone (*stratum germinativum* of the penile epithelium) is similar in structure to the outer zone. Where the frenulum is thin the cells of the apposed germinal zones become cuboidal and merge with the middle zone (Pl. 1, fig. 1).

The middle zone is a mass of cells joining the inner and outer zones. The nuclei stain less intensely than those of the germinal zones. Keratinization is found in the middle zone of the lamella in the young bull calf. Generally it is concentrically developed from small balls of prickle cells, resulting in the formation of epithelial pearls. Longitudinal keratinization is less common. Where keratinization occurs there is a tendency for the cells of the middle zone to clump together, forming cigar-shaped basophilic masses of four to twelve nuclei. These 'clumps' are often arranged irregularly around the periphery of the epithelial pearls (Pl. 1, fig. 4) and occur only at the sites of keratinization. In these calves keratinization was almost entirely restricted to apical levels. The thick middle zone of the lamella around the urethral process was usually riddled with epithelial pearls (Pl. 1, fig. 2) and pearls often occurred in the arms of the lamella bordering the frenulum. Inner and outer zones appear not to be affected by the keratinization except where large epithelial pearls bulge the outer zone into the tissues of the sheath.

(2) *The frenulum*

The frenulum extends over the basal five-sixths of the free end of the penis. Its shape, as seen on transverse section, varies at different levels and four distinct segments can be distinguished:

(*a*) An *apical segment* where the left arm of the lamella overlaps the right arm and is bent back on itself so that the inner zones of the right and left arms are apposed and the frenulum is oblique (Text-fig. 1 D).

(*b*) A *transitional segment* where either the two arms do not overlap and the frenulum is vertical or one or both arms are forked and by their overlapping delimit a forked frenulum (Text-fig. 1E).

(*c*) A *basal segment* where the overlapping is reversed: the right arm overlaps the left, its outer zone lies apposed to the inner zone of the left arm, and the frenulum is oblique (Text-fig. 1F).

(*d*) Where the dorsal diverticulum of the sheath extends over the base of the free end the frenulum becomes wider, passing dorsally and caudally along the caudal edge of the sheath at its point of reflexion (Text-fig. 1G, H).

The relative lengths of these segments vary between specimens. Apical and basal segments both occupy about one-third to one-quarter, the transitional segment one-sixth, and the dorsal sac of the sheath one-tenth, of the total length of the free end. The transitional segment occurs near the base of the terminal swelling.

The frenulum is a thin band of connective tissue, predominantly collagenous. It is irregular in shape and size; in a transverse section its minimum width is usually 20–30μ. However, in two of these calves the frenulum was considerably wider for 3–4 mm. of the apical segment; the minimum width was in some sections 300μ. In both cases a vein passed through the frenulum at this point forming an anastomosis between the veins of the corpus cavernosum urethrae and the sheath.

(3) *The foetal ectodermal lamella*

In the smallest foetus (135 mm.) the transformation of the *penis pendulus* into a *penis appositus* had only just been completed. At the apex of the penis the epithelia of penis and sheath were separate, but over the basal two-thirds of the free end they were joined, forming an ectodermal lamella. This was very thick in parts, and not so clearly divided into zones as in the calves, though over the apex of the free end the cells of the inner zone were columnar and very distinctive. These cells were similar in the 23 and 26 cm. foetuses. In all but the two largest foetuses the urethral process lay close to the ventral wall of the sheath and ventrally the lamella was very thin (Text-fig. 2A).

Keratinization was seen in the ectodermal lamellae of all these foetuses except the 135 mm. specimen. Epithelial pearls in the 23 cm. foetus were small and infrequent; in the largest foetuses (70, 73 cm.) they were less well developed than in the born calves and were mainly found alongside the urethral process and the frenulum. Nuclear ‘clumps’ were infrequent.

(4) *The foetal frenulum*

In the 135 mm. foetus there was no overlapping of the arms of the lamella; the frenulum was vertical throughout its length and very wide near the apex of the penis. In the other foetuses the shape of the frenulum varied at different levels in the same way as in the young calves. Overlapping of the arms of the lamella was most marked in the largest foetuses. In many places the arms of the lamella came very close together, and the frenulum was reduced to a single layer of connective tissue cells. Over a length of 500μ the frenulum of the 49 cm. calf was exceptionally wide, reaching a minimum width of 130μ. A large vein, anastomosing between the veins of the corpus cavernosum urethrae and those of the sheath, traversed the frenulum at this point.

DISCUSSION

In general, this examination of the penis and sheath of foetal and young calves agrees with the descriptions of embryological development of the external genitalia of ruminants given by Retterer (1890, 1915) and Böhm (1905).

During the first half of pregnancy the ectodermal lamella shows evidence of the keratinization that eventually splits it into two epithelial surfaces. These cell-changes are very similar to those described in the human lamella by Stieve (1930) and Deibert (1933), but these authors do not describe the nuclear 'clumping' that is so noticeable in the lamella of the young calf. Keratinization is well established at the tip of the penis in the calf shortly after birth, but there is no macroscopical separation at this stage and the penis does not become completely free until the bull is nearly one year old (Ashdown, 1959). It has been shown in many mammals that keratinization of the ectodermal lamella is controlled by testicular hormone (Wiesner, 1934; Raynaud & Lacassagne, 1937; Lyons, Berlin & Friedlander, 1942; Wells & Lund, 1947) and this is almost certainly so in the ruminants (Retterer & Neuville, 1916; Wiggins & Terrill, 1953). The keratinization of the lamella in these foetuses seems indicative of androgenic stimulation during the second half of pregnancy.

During separation of the bovine penis from its sheath the frenulum ruptures; fragments of tissue remain, however, forming the raphe on the penis and sheath of the adult bull. Fleischmann (1907) and Böhm (1905) both believed that the frenulum of the domesticated ruminants is obliterated before birth by the active growth of the ectodermal lamella. Retterer (1890) agreed with this, but later (1915) stated that the obliteration is not completed till after birth. My material shows clearly that the frenulum of the ox persists into postnatal life. However, the progressive overlapping shown by the arms of the lamella during the second half of pregnancy suggests some active growth of the cells of the lamella and seems to support the view of Fleischmann, Böhm and Retterer on the dynamic role of ectodermal cells in development of this region. The early and extensive appearance of keratinization along the edges of the lamella abutting the frenulum suggests the possibility that this too may play some part in rupturing the thin band of connective tissue. Increased width of the frenulum at those places where a traversing blood vessel occurs could, perhaps, affect the process of frenular rupture in some young bulls.

SUMMARY

Penis and sheath have been examined in young calves up to 3 weeks of age and in foetuses ranging in size from 135 mm. to 73 cm. C.R. length. In these specimens the penis was united to the penile sheath by a lamella of ectodermal cells and a frenulum of connective tissue: these structures are described. Keratinization, which separates penis from sheath in later life, was proceeding in the ectodermal lamella of each specimen except the smallest foetus.

Most of this work was done during my tenure of an A.R.C. Research Studentship, under the supervision of Prof. C. W. Ottaway. Dr E. H. Batten gave me much advice in the preparation of this paper and took the photomicrographs. Miss P. Morgan helped me with the histological preparations.

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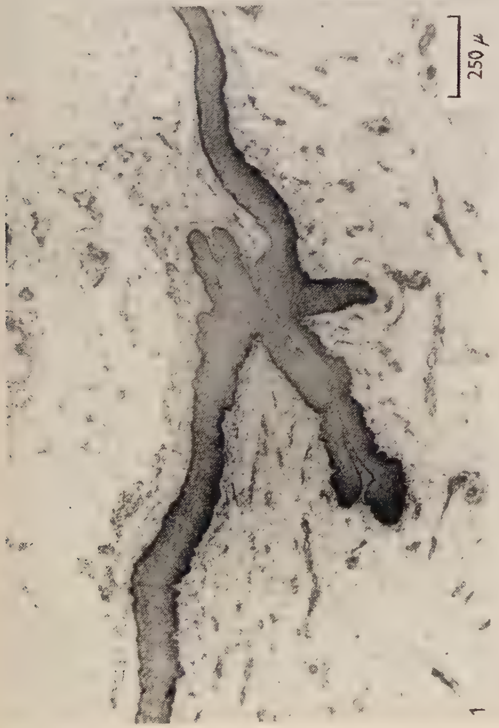
EXPLANATION OF PLATE

Fig. 1. Transverse section through penis and sheath of a young bull calf, passing through the apical segment of the frenulum. (Formal saline; 10μ; H. & E.) The frenulum is thin and oblique. Where the ectodermal lamella borders the frenulum the apposed inner zones are almost indistinguishable from the middle zones.

Fig. 2. Transverse section through urethral process and sheath of a young bull calf showing extensive keratinization of the ectodermal lamella. (Bouin; 10μ; H. & E.)

Fig. 3. A higher power view of a section adjacent to that of fig. 2, showing the structure of the ectodermal lamella. The columnar cells of the outer (*OZ*) and inner (*IZ*) zones have intensely staining nuclei lying at right angles to the basement membranes. The nuclei of the middle zone (*MZ*) stain less intensely. At this point three epithelial pearls lie in the middle zone.

Fig. 4. A higher power view of a field adjacent to that of fig. 3, showing two large epithelial pearls. In the centre of each is a cellular eosinophilic mass. The surrounding cells are swollen, with faintly staining cytoplasm, eosinophilic granules and pycnotic nuclei. Arrows indicate some of the places where nuclei have clumped together forming characteristic basophilic masses around the periphery of the epithelial pearls.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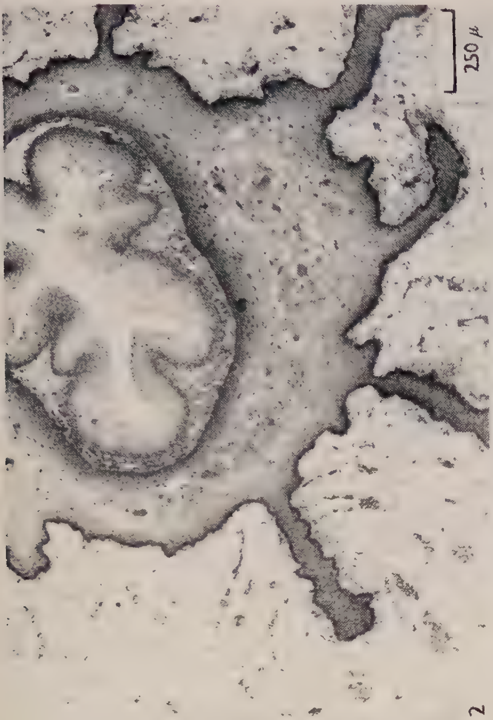
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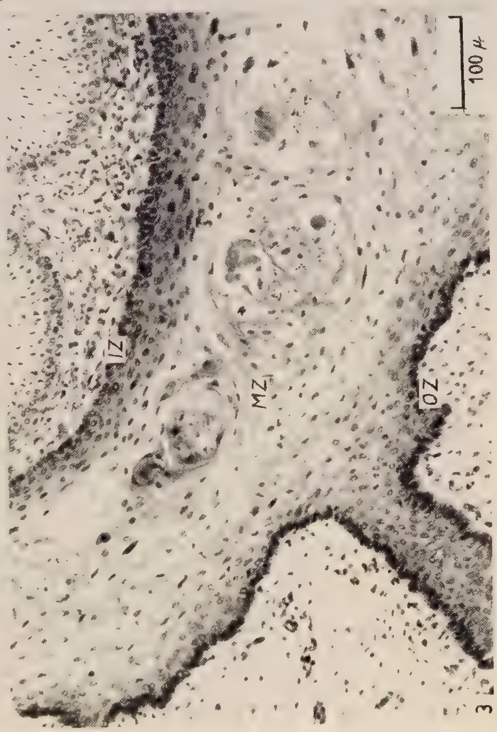
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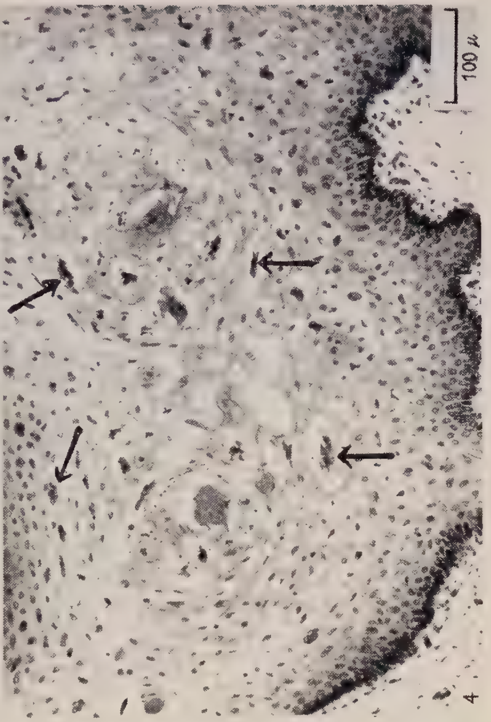
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ASHDOWN—THE ADHERENCE BETWEEN THE FREE END OF THE BOVINE PENIS AND ITS SHEATH

(Facing p. 204)

ELECTRON MICROSCOPIC OBSERVATIONS ON THE VILLOUS HAEMOCHORIAL PLACENTA OF THE NINE-BANDED ARMADILLO (*DASYPUS NOVEMCINCTUS*)

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INTRODUCTION

Studies of the fine structure of placentas of the haemochorial type have been made by Dempsey (1953, 1954), Boyd & Hughes (1954), Wislocki & Dempsey (1955*a*, *b*), and Sawasaki, Mori, Inoue & Shinmi (1957). Electron microscopic observations of placentas of other types have been reported by Dempsey, Wislocki & Amoroso (1955), Dempsey & Wislocki (1956), Amoroso & Matthews (1958), and Wimsatt (1958). Examination of the labyrinth of the rat placenta revealed not only that the trophoblast is present through day 21 of gestation, but also that it is cellular rather than syncytial (Wislocki & Dempsey, 1955*a*). Studies of the villous haemochorial placenta of the human by Boyd & Hughes (1954), Wislocki & Dempsey (1955*b*), and Sawasaki *et al.* (1957) have confirmed the belief that the trophoblast covering the vascular villi is syncytial and have also demonstrated that individual Langhans cells may persist until term. The nature of the syncytium was especially clearly elucidated by the studies of Wislocki & Dempsey (1955*b*) who found that the syncytium was characterized by the presence of numerous microvilli and frequent amorphous vacuoles in addition to the usual organelles.

Recent histological studies concerning placentation in the armadillo (Enders, Buchanan & Talmage, 1958; Enders, 1959) have shown that the epithelium of the vascular villi is syncytial and possesses a variable free surface. These studies also indicate that the syncytial trophoblast is derived from the cytotrophoblast and that both the cell columns and the modified fibroblasts seen in the stroma of mature vascular villi may be glandular. In the electron microscopic study reported here special attention has been paid to (1) the nature of the placental barrier, (2) the organization of the syncytium, and (3) cytological evidence of glandular activity.

MATERIALS AND METHODS

Pregnant female armadillos were killed by abrupt fracture of the vertebral column. The uterus was excised immediately and cubes of placenta of 2 mm. or less were fixed in Palade's (1952) veronal acetate buffered osmium tetroxide for ½–1 hr., or in Dalton's (1955) chrome osmium fixative for 1 hr. Following fixation the blocks of tissue were dehydrated in several changes of ethyl alcohol and embedded in methacrylate, and sections were made on a Porter–Blum mechanical advance microtome. Initial examination of the material was made using an RCA–EMT electron microscope. Electron micrographs of the better specimens were made with an RCA–EMU 3B electron miscrope. Other portions of the placentas were fixed for routine histological preparation. If the histological preparations did not indicate that the placenta was normal for its stage of development, the methacrylate-embedded sections were rejected. In all, placentas from seven animals were used: three from the period of initial invasion, three from the period of expansion of the placenta, and one from the period of the mature placenta. Implantation was induced by bilateral ovariectomy in the three cases collected from the period of placental invasion. The other placentas came from animals from the feral population. In addition to the placental material, five blastocysts from animals in the period of delay of implantation were prepared by the procedures described above.

DESCRIPTION

Syncytium

The placental barrier in the villous haemochorial placenta of the armadillo consists of the trophoblastic epithelium, an underlying basement membrane, and the endothelium of the foetal capillaries. Study of the electron micrographs of vascular villi reveals that the trophoblast covering these structures is truly syncytial (Pl. 1, fig. 1). Its free surface is characterized by the presence of numerous, branched, lamelliform microvilli (Pl. 2, figs. 4–6). The number, nature, and complexity of the microvilli vary within a single placenta, as might be expected from observations with the light microscope. The microvilli appear to be folds in the surface membrane of the syncytium. In areas of greater complexity they are not simple extensions but rather are projections from protruding masses of cytoplasm which extend into the intervillous space. At its base each fold is long and usually curved, becoming narrower at its distal apex, which is rounded. Secondary folds are continuous with the initial fold. Although the length from the surface and extent along the surface vary, the thickness of the folds is relatively constant. The result of this arrangement of the microvilli is an uneven surface composed of folds of the cytoplasmic membrane which terminate distally at simple rounded projections, with cup-like spaces between the bases of the folds. The complexity of these microvilli is clearly revealed in Pl. 1, figs. 2 and 3 and Pl. 2, fig. 6. The microvilli are somewhat shorter and more numerous in the mature placenta.

The total thickness of the syncytium is increased at the locations of the nuclei. Although there is ordinarily only a thin layer of cytoplasm which is relatively free of microvilli covering the distal aspect of the nuclei, there is always an appreciable thickness of cytoplasm separating the nuclei from the basement membrane. The nuclei are characterized by the presence of a single, large nucleolus.

Within the cytoplasm of the syncytium are numerous rod-like mitochondria with well-defined cristae. The cristae are not in a highly ordered parallel arrangement but are more commonly villiform. Mitochondria are more numerous near the free surface of the syncytium, and are frequently oriented parallel to it. A few are also associated with the basal cytoplasm. The mitochondria do not appear to alter greatly in structure or number from the period of placental expansion to the period of the mature placenta.

The endoplasmic reticulum is largely confined to the distal cytoplasm. During the period of placental expansion double strands of reticulum are commonly found in concentric circles within protoplasmic projections into the intervillous space (Pl. 1, fig. 1). In the juxtanuclear cytoplasm and other areas beneath the general level of the surface of the syncytium, occasional double strands of reticulum are seen in addition to numerous endoplasmic vesicles or branched cisternae. The form of the reticulum varies considerably; strands in the promontories and cisternae in the distal cytoplasm are most commonly encountered. The electron-dense particles of the particulate component (Palade's particles, presumably ribonucleoprotein) are found free in the cytoplasm as well as associated with the reticulum. In the mature placenta protoplasmic projections into the intervillous space are less frequently encountered, and the endoplasmic reticulum is characteristically in the form of dilated cisternae which contain an amorphous material of moderate electron density.

The membranes and vesicles of the Golgi complex are located at the basal margin of the region of the syncytium in which endoplasmic cisternae are found. In other words, they are directly distal to the morphologically distinct basal cytoplasm of the syncytium. Although the individual regions of Golgi membranes lie at the same level within the syncytium, there is no evidence that they are continuous. The form and extent of these regions are comparable to those of non-syncytial glandular cells. The increase in cytoplasmic density and in number of ribonucleoprotein granules found in the cytoplasm of the syncytium of the mature placenta makes the Golgi complex difficult to discern at this stage, but its relative position remains the same.

Beneath the Golgi membranes is an area of cytoplasm containing canaliculi formed by the invagination of the basal membrane of the syncytium (Pl. 1, fig. 2). The appearance of the canaliculi in sections indicates that they are tortuous and probably branched. In later pregnancy the canaliculi are more extensive and may be in partially dilated or non-dilated form (Pl. 2, fig. 5). Filamentous structures are frequently found within this area of the cytoplasm, giving it a fibrillar appearance.

Stroma

The basement membrane which underlies the syncytium is composed of a feltwork of an amorphous material of moderate electron density and a few collagen fibres (Pl. 1, fig. 2). The stroma of vascular villi contains a few collagen fibres in the period of placental expansion. In the mature placenta, however, collagen fibres are a conspicuous component of the basement membrane and the connective tissue in general.

The endothelial cells forming the foetal capillaries are relatively unspecialized except for the occasional microvilli and the frequent presence of lipid droplets. At no time in pregnancy are discontinuities or areas of especial thinness found in the endothelial cells (Pl. 2, fig. 1).

The fibroblasts have irregular outlines with numerous processes which may abut on adjoining fibroblasts. There is no indication that the cytoplasm of these cells is ever continuous. The cytoplasm of the fibroblasts contains a few mitochondria and strands of endoplasmic reticulum with frequent vesiculations. The ground substance of the cytoplasm is not very electron-dense in the early stages. The Golgi membranes are inconspicuous. The mature placenta, however, contains numerous highly modified fibroblasts. These are rounded cells containing a vast array of dilated endoplasmic cisternae. The extent of dilation is somewhat variable. Within the cisternae is a finely granular and moderately electron-dense substance. Ribonucleoprotein granules are abundant and are clearly associated with the membranes of the reticulum which completely fills the cytoplasm except in the region of the Golgi complex and in small areas where a specific granulation is observed (Pl. 5, fig. 12). The Golgi complex of these cells occupies an area nearly as large as that of the nucleus and is primarily composed of numerous concentric strands of Golgi membranes (Pl. 5, fig. 11). Contained within this region are a few vesicles derived from the reticulum, Golgi vesicles, mitochondria, and unidentified background substance.

Cell columns

Cell columns form the proliferating tips of the vascular villi from which they are separated by a layer of syncytium (Pl. 3, fig. 7). The syncytium overlying the free surface of the cell columns is exceedingly thin, but in all instances observed it forms a complete covering. Between the cells of the cell columns are numerous spaces (Pl. 3 fig. 8). Within the individual cells lipid droplets are common and mitochondria are present but are not especially numerous. A scant reticulum is generally present in addition to the numerous ribonucleoprotein granules that are seen free or in clusters in the cytoplasm. Occasional lipid droplets are surrounded by concentric rings of reticulum. The Golgi complex is poorly developed in these cells. Within the intercellular spaces large accumulations of a granular substance of moderate electron density are seen. In the regions abutting on the intercellular masses the cell membranes are occasionally indistinct, and a granulation similar in density to that of the intercellular granules is discernible in the adjacent cytoplasm. In no instances was a basement membrane observed underlying the syncytium covering the cell columns. However, where the cells of the cell columns are in close association with the syncytium, desmosomes (terminal bars, adhesion zones) are present, and associated filaments (tonofibrils) can be seen in the cytoplasm (Pl. 3, fig. 7; Pl. 4, figs. 9, 10). The thin syncytium overlying the cell columns frequently contains vacuoles, in some of which granular material of identical appearance to that of the intercellular substance is seen. This overlying syncytium displays great irregularity in thickness. Although microvilli are common in some areas, they are sparse in others and never show the complexity seen in other portions of the placenta. The desmosomes vary in area from small points of contact to complete sheaths surrounding a projection into the syncytium. Frequently the paired adhesion plates of the individual desmosomes are readily apparent (Pl. 4, fig. 10).

Preliminary observations on early implantation

Blastocysts and early implantation stages proved to be more difficult to fix than did the placenta of the expansion period and the mature placenta. Nevertheless, preliminary observations of these stages are of considerable interest. The abembryonic trophoderm of the blastocyst is composed of a single layer of epithelial cells without any indication of internal or external supporting membranes (Pl. 6, fig. 16). The adjacent cell surfaces interdigitate only toward the basal end of the cells. These interdigitations are not the peg or tongue-in-groove type commonly present in epithelia (Fawcett, 1958) but are plaque-like extensions of the cytoplasm. The basal plasma membrane (fronting on the blastocoel) of the cells is relatively smooth, but microvilli are present on the convex distal surface. The mitochondria are most numerous near the surfaces of the cells, but a few are also found near the basal membranes. Our observations are insufficient to permit description of the other organelles.

The invasive trophoblast is characterized by the presence of numerous small vesicles of undetermined origin, and fat droplets. The cytoplasm is not very electron-dense, and ordinarily only scattered mitochondria are discernible. The surfaces of the tongues of invasive syncytium frequently display numerous simple microvilli. These microvilli are confined to areas where the trophoblast is free from contact with epithelial cells. The tongues of syncytium are most frequently seen in the connective tissue between endothelial cells and the glandular epithelium of the endometrium (Pl. 6, fig. 17).

Normal glandular epithelial cells of the endometrium have large oval nuclei, with an uninterrupted, convex outline (Pl. 6, fig. 13). The basal membrane of these cells is relatively unmodified. The membranes forming the walls of the individual columnar epithelial cell form peg-like interdigitations with adjacent cell membranes, especially at the angles where three cells are in contact. Terminal bars are characteristically present at the luminal surface which has numerous simple microvilli. A few epithelial cells are ciliated. Unmodified epithelial cells contain small lipid droplets, numerous mitochondria, a few strands of reticulum, and a distal juxta-nuclear Golgi region. The modification of the epithelial cells that occurs at implantation is characterized by hypertrophy of the individual cells and the accumulation within these cells of numerous large vesicles, some of which appear to be derived from the reticulum (Pl. 6, fig. 14). A fine precipitate can be discerned within many of the larger vesicles (Pl. 6, fig. 15). The only cytoplasmic inclusion sufficiently abundant to account for the amount of material suggested by these larger vesicles is glycogen (Enders *et al* 1958). No enhancement of the Golgi membranes has been observed and there is no apparent increase in the number of mitochondria. With increase in cell size the microvilli at the free surface become stubby or disappear. Degenerating epithelial cells in the area of invasion apparently become highly vesiculate and lose much of their electron density.

DISCUSSION

The placental barrier, as determined by a study of electron micrographs, consists of the syncytial trophoblast, the basement membrane, and the foetal endothelial cells. It is doubtful that the connective tissue between the basement membrane and the endothelial cells constitutes a significant part of this barrier. The basement membrane is clearly a product of the stroma, since there is no basement membrane underlying the syncytium covering the cell columns.

The syncytium itself exhibits considerable variation both in the extent of microvilli and in the amount and form of derivatives of the endoplasmic reticulum. It might be argued that the variation exhibited is a result of unequal quality of fixation. This does not seem to be the case, however, for adjacent villi show variations in structure within the zone of better fixation. Furthermore, variation in structure of the syncytium is also common in ordinary histological preparations preserved with fixatives that act more uniformly than does osmium tetroxide. It has not been possible, however, to determine whether these variations in syncytial structure are randomly localized or are characteristic of different portions of the villous tree.

A comparison of the syncytium of the armadillo with that of the human, as reported by Wislocki & Dempsey (1955*b*), reveals that there are many features in common. Both animals exhibit thinning of the syncytium in older placentas. Microvilli which may arise from protoplasmic protrusions are common to both, as are endoplasmic vesicles. However, neither the complexity of microvilli nor the polarity of intracellular organelles observed in the armadillo syncytium has been reported in the human. Furthermore, there is very little lipid in the syncytium of the armadillo except in the invading trophoblast while lipid droplets are a common feature of the syncytium of the human placenta (Wislocki & Dempsey, 1955*b*). The infolding of the basal cytoplasmic membrane to form canaliculi, which is particularly characteristic of later placentas in the armadillo, has not been reported for the human placenta. The presence of numerous vacuoles and the extent of variation of the free surface of the syncytium were considered by Boyd & Hughes (1954) and Wislocki & Dempsey (1955*b*) to be evidence of pinocytosis. The relative uniformity and complexity of microvilli and the lack of vacuoles in the syncytium of the armadillo placenta suggest a relatively stable surface with a greatly increased area as opposed to a labile surface characterized by pinocytotic activity.

The association of the mitochondria with the region of the cytoplasm in which the microvilli arise is reminiscent of the more intimate association of mitochondria with the folding of the basal plasma membrane in the convoluted tubule of the kidney and ependymal cells (Pease, 1956). It is interesting to note that the Golgi membranes are disposed towards the basal region of the syncytium. The Golgi complex in glandular cells is generally situated in a distal juxtanuclear position, i.e. between the nucleus and the secretion surface. The implication, therefore, is that as a secretory organ the syncytium should be viewed as being based on the intervillous space with its apex on the basement membrane, an orientation compatible with its function. The tortuous canaliculi formed by the basal plasma membrane serve to increase the surface available for exchange of materials between the syncytium and the stroma of the villi just as the microvilli increase the surface available for exchange between the syncytium and the intervillous space. Why the canaliculi should take the particular form which they do is not immediately apparent.

It might be argued that the substance seen in the intercellular spaces of the cell columns was derived directly from the maternal blood vascular system rather than being a secretion product of the cytotrophoblast. There are two principle arguments against this possibility. In the first place, there are regions within the cytotrophoblast cells which demonstrate the same sort of granulation as the substance in the intercellular space. In addition, this substance is found in vacuoles within the syncytium overlying the cytotrophoblast. This latter observation might indicate that the secretion product was passing in either direction, except that it is found only in the vacuoles in the syncytium overlying the cell columns and never in the vacuoles in the syncytium covering the vascular villi. It does not seem probable that the syncytium would be phagocytic or pinocytotic only where it covers the cell columns. Furthermore, the syncytium is formed, in all probability, from the cells of the cell columns (Enders, 1959). Thus the substance liberated into the intercellular spaces is transported to the intervillous space during the process of formation of the syncytium from the cytotrophoblast. It is interesting to note that this material has the histochemical characteristics of a glycoprotein (Enders, 1959). It remains to be demonstrated that the armadillo placenta produces gonadotropins.

The individuality of smooth muscle cells, mesenchymal cells, stratified squamous epithelial cells, and the units of cardiac muscle between intercalated discs has been disclosed by electron microscope studies (see Fawcett, Ito & Slautterback, 1959, for a survey of the extensive literature). A previously undescribed form of syncytium which is formed by incomplete separation of cells at telophase has been described for cnidoblasts of *Hydra* and mammalian spermatids by the above authors. The remaining examples of syncytia, with the possible exception of skeletal muscle fibres, are all of the type which develops by coalescence of previously individual cells. The fusion of individual cells to form these syncytia (multinucleate masses, giant cells, osteoclasts, syncytial trophoblast) has yet to be described.

Fawcett (1958), in his review of the nature of the cell membrane, has pointed out the frequency with which thickenings occur in the cytoplasm at areas of contact of cell membranes forming desmosomes. Intercalated discs (Fawcett & Selby, 1958), terminal bars, nodes of Bizzozero, and other specific regions of cytoplasmic contiguity have all been interpreted as modifications of the type of structure which is designated desmosome. Recently Odland (1958) has studied in greater detail the organization of these structures in human epidermis. In addition to the fibrillar element (tonofibrils) and attachment plaques (paired areas of increased density within the cytoplasm), he was able to discern intervening layers of unidentified substances occupying the space between attachment plaques. All of the studies of desmosome structure have emphasized that there is no continuity of the cytoplasm through the attachment plaques.

One of the interesting and unexpected observations concerning the armadillo placenta was that where cells of the cytotrophoblast came in close association with the syncytium, desmosomes were formed. These desmosomes are most frequently found in areas in which there are numerous vacuoles containing intercellular substance within the syncytium. Frequently the desmosomes are small areas of contact or form relatively flat regions of contiguity between the cytotrophoblast and the syncytium. The attachment plaques and fibrils are most clearly distinguished, however, in regions where there is considerable interdigitation of processes of the cells of the cell columns with the overlying syncytium. It therefore seems that the formation of the desmosomes represents an initial stage in the transformation of a cell from the cell column into a portion of the syncytium. It is reasonable that the close association of the cell membranes over a considerable surface should constitute a preliminary to fusion of the cytoplasm. However, it is rather surprising that there should be an initial increase in density of the adjacent cytoplasm prior to breakdown of the cell membrane.

That the endoplasmic reticulum is a system of membranous components with which granules of high electron density may be associated is clearly established (see reviews of Palade, 1958, and Robertson, 1959). The particulate component has been demonstrated to contain ribonucleoprotein (Palade & Siekevitz, 1956; Palade, 1958). Additional evidence currently accumulating indicates that, while the large numbers of ribonucleoprotein particles characteristic of proliferating cells and other cells with a high rate of synthesis of cytoplasm are not associated with endoplasmic membranes, an extensive system of endoplasmic membranes with associated ribonucleoprotein particles is characteristic of cells synthesizing specialized proteinaceous cell products (Slautterback & Fawcett, 1959). Since the cisternae represent a space which is isolated from the cytoplasm, although not necessarily from the nucleus (Watson, 1955) or the exterior (Epstein, 1957), it has been suggested by numerous authors that these spaces are areas where intermediate or final products of synthesis may be accumulated (Siekevitz & Palade, 1958; Slautterback & Fawcett, 1959). The pattern of formation of the nematocyst of *Hydra* suggests to the last-mentioned authors that the specific proteins synthesized were accumulated, probably in an intermediate form, in the cisternae of the endoplasmic reticulum. The formative material then is accumulated within vacuoles in the Golgi complex, a pattern of development which is similar to acrosome formation (Burgos & Fawcett, 1955). Hally (1958) drew somewhat similar conclusions with regard to the Golgi complex from his study of Paneth cells.

The Golgi zone is admirably positioned for the accumulation of materials resulting in the formation of an intracellular body. However, its juxtanuclear location does not seem consistent with the accumulation of the end products of synthesis in most gland cells. Palade & Siekevitz (1956) and Siekevitz & Palade (1958) have presented evidence that pancreatic secretion granules are synthesized by the ribonucleoprotein granules, segregated within the cisternae of the endoplasmic reticulum and, as zymogen granules, may retain a membranous covering derived from this structure.

The modified fibroblast cells of the mature placenta have a very extensive endoplasmic reticulum with which numerous ribonucleoprotein granules are associated. In addition, the cisternae are dilated and contain a granular substance of moderate electron density. Larger granules are present in the cytoplasm at the confluence of a number of cisternae. As in other glandular cells, the Golgi complex is highly developed. These observations and those with the light microscope are typical for a highly differentiated protein secreting cell. In addition the specific organization of the organelles of these cells suggests the following speculative interpretation: metabolites are accumulated in the Golgi complex; protein is synthesized by the ribonucleoprotein granules, the intermediate products accumulating in the cisternae; the protein granules assume final form in the cytoplasm. The arrangement of the cisternae suggests not only accumulation of intermediates, but also that they serve to transport the substance to the site of final formation.

Our investigations on the invasive trophoblast are preliminary. However, what little evidence we have garnered indicates that the trophoblast penetrates into the connective tissue of the stroma between endothelial and epithelial cells. Electron microscopy should be an excellent means of studying the initial penetration of the blastocyst into the endometrium. Studies of this nature are currently being undertaken.

SUMMARY

The placental barrier, as seen in electron micrographs, is composed of the syncytial trophoblast and the mesodermal core of the vascular villus. The syncytium has numerous elongate microvilli on its free surface. The microvilli are especially well developed in some regions of the placenta where they constitute numerous, branched, lamelliform projections into the intervillous space. A distinct zonation of intercellular elements is apparent, with the endoplasmic reticulum and mitochondria being situated largely in the distal and juxtanuclear cytoplasm and the Golgi in a more proximal position. It is suggested that this orientation constitutes a reversal of the apparent polarity of the syncytium. The basal plasma membrane is invaginated forming branched canaliculi in the basal cytoplasm which often contains small fibrillar elements. The stroma of the vascular villi is composed of the basement membrane, the connective tissue elements, and the endothelial cells of the foetal capillaries. It is doubtful that the finely fibrillated intercellular space of the stroma constitutes much of a barrier. The endothelial cells of the foetal capillaries possess neither perforations nor thin areas. The fibroblasts in late pregnancy are modified and contain an extensive endoplasmic reticulum composed of dilated vesicles with numerous associated ribonucleoprotein granules. In addition, these cells have a large region of Golgi membranes and a specific granulation in their cytoplasm. It is suggested that they are protein-secreting elements.

The syncytial trophoblast overlying the cell columns is especially thin, and no basement membrane is found in this region. Desmosomes are present, however, where the cells of the cell columns are in intimate contact with the syncytium and may constitute an initial stage in fusion of the adjacent cytoplasm. Within the intercellular spaces in the cell columns is a granular substance. It is suggested that this is a secretion product of the cytotrophoblast which is liberated into the intervillous spaces during the formation of syncytium from the cells of the cell column.

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EXPLANATION OF PLATES

These symbols are used in the following figures: *BM*, basement membrane; *D*, desmosome; *END*, endothelium; *EP*, epithelium; *ER*, endoplasmic reticulum; *ERC*, cisternae of the endoplasmic reticulum; *ERYT*, erythrocyte; *F*, fibrils; *FC*, foetal capillary; *GC*, Golgi complex; *Gr*, intercellular granulation; *L*, lipid; *N*, nucleus; *NCL*, nucleolus; *M*, mitochondria; *MC*, maternal capillary; *MBS*, maternal blood space; *S*, intercellular space; *TR*, trophoblast. All magnifications are approximate.

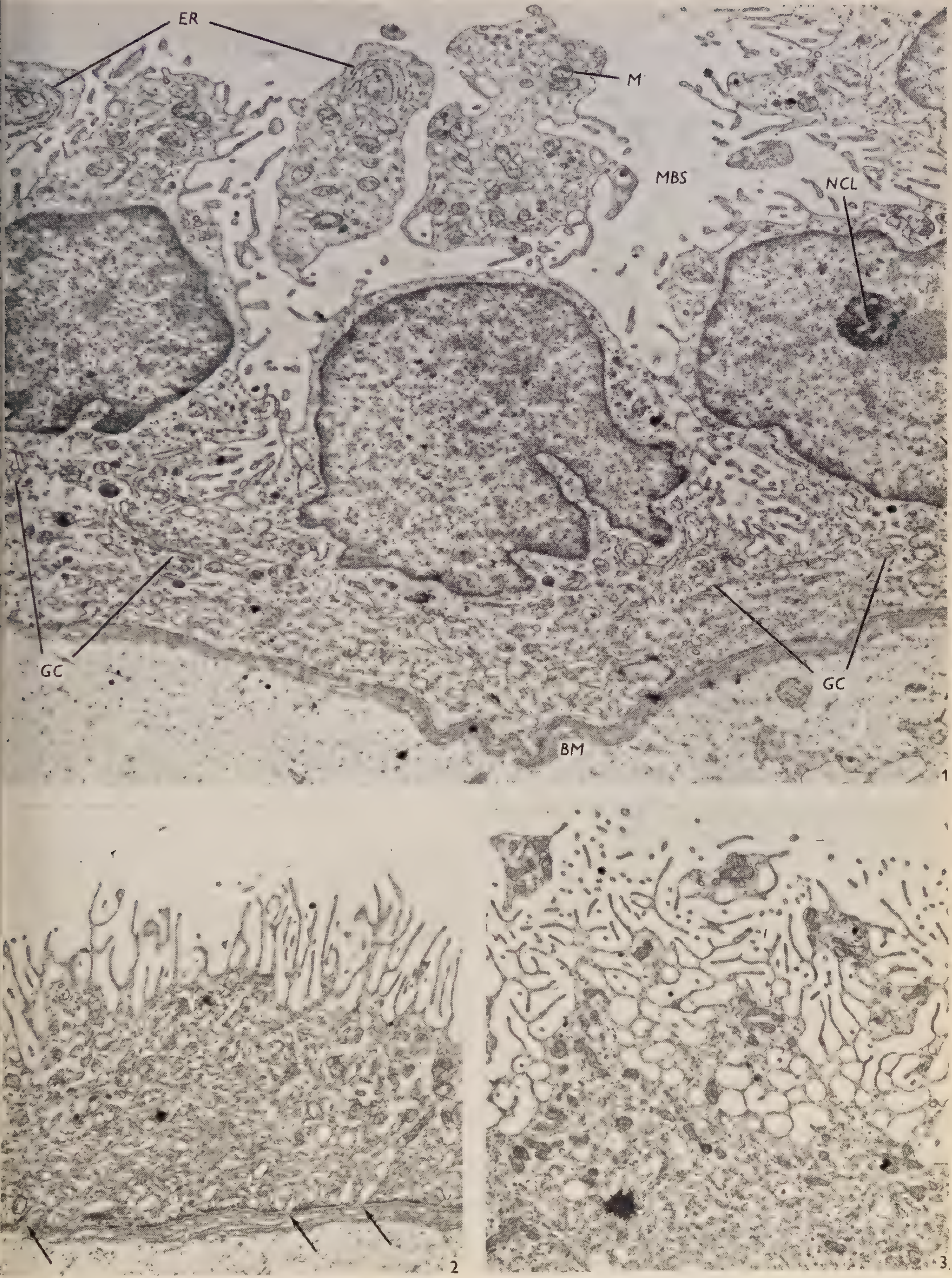
Plate 1

Structure of the syncytial epithelium of the vascular villi
during the period of placental expansion

Fig. 1. Syncytial trophoblast from the period of placental expansion. Although there are parts of four nuclei visible, there are no plasma membranes between these nuclei. Both microvilli and protoplasmic projections are illustrated. A section in which the internuclear distance is much less than usual was selected. The zonation of organelles within the syncytium is readily apparent. × 5700.

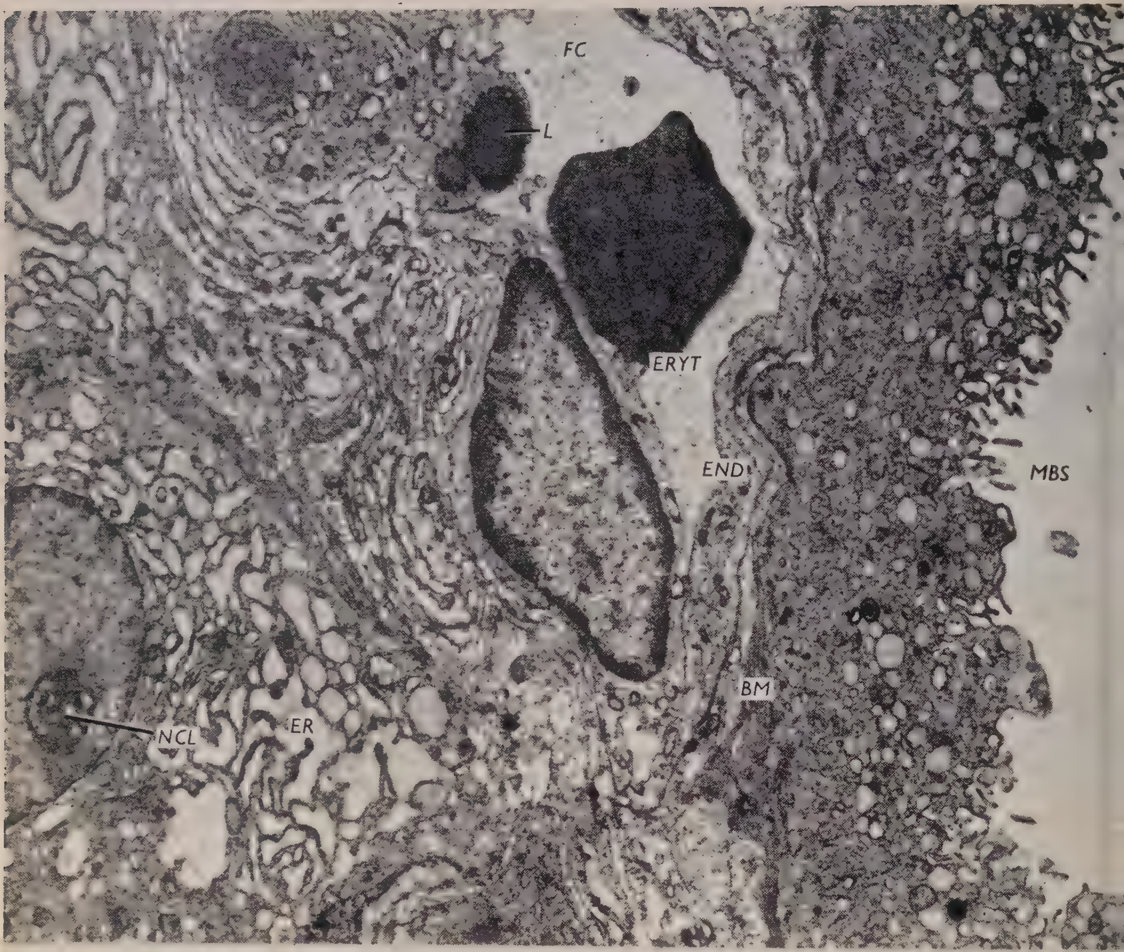
Fig. 2. A portion of the syncytium from the stage of placental expansion. The infolding of the basal plasma membrane to form the canaliculi is seen at the arrows. × 7600.

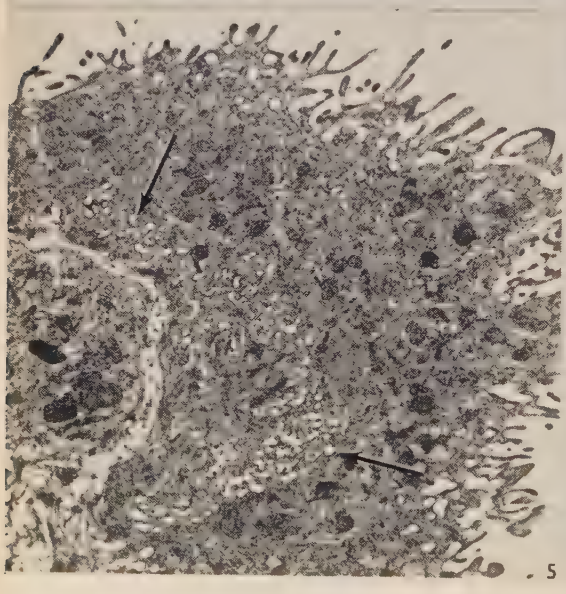
Fig. 3. A tangential section of the surface of the syncytium. Note that the lamellar microvilli have a relatively uniform thickness, and that in areas of this complexity cups are formed between the microvilli. × 7600.

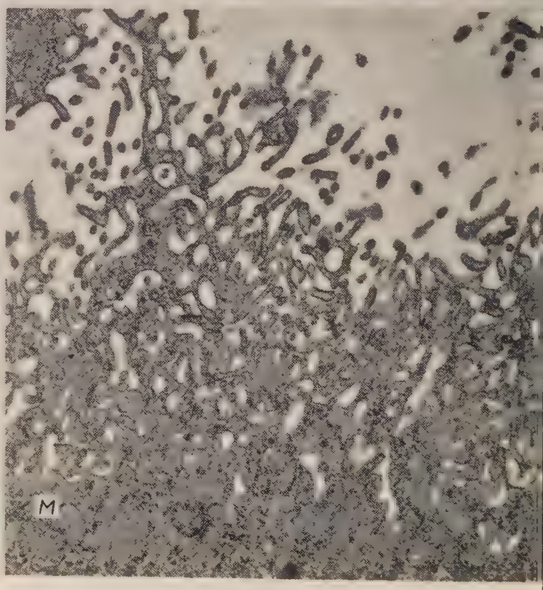


ENDERS—Electron microscopic observations on the villous haemochorial placenta of the nine-banded armadillo (*Dasypus novemcinctus*)

(*Facing p.* 214)







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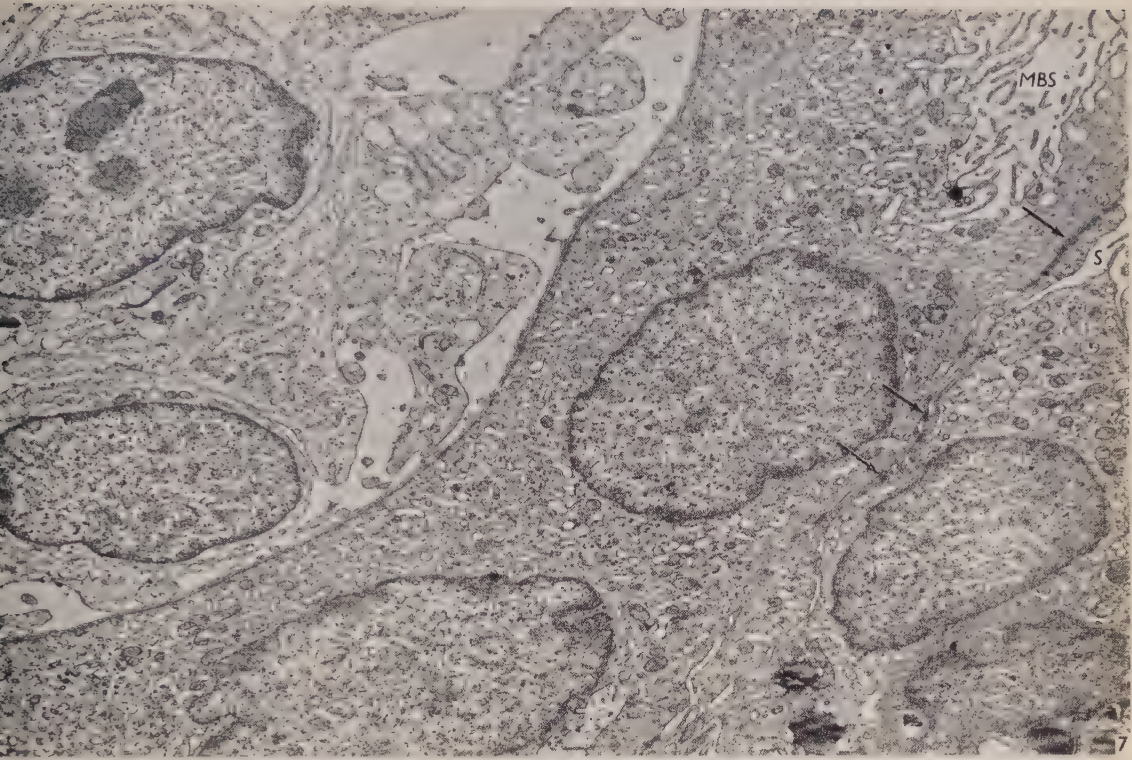

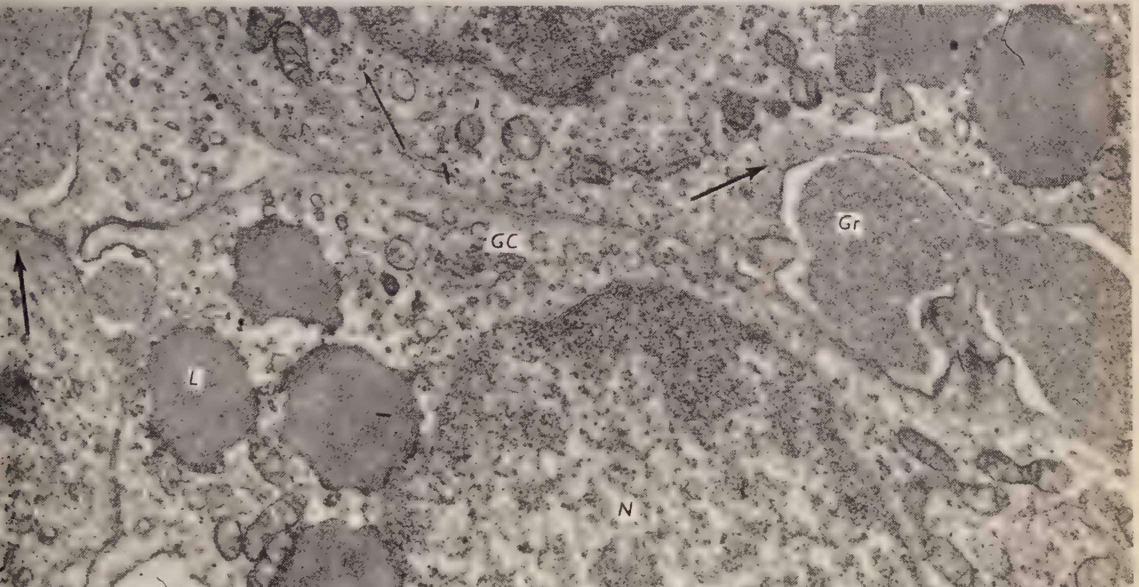

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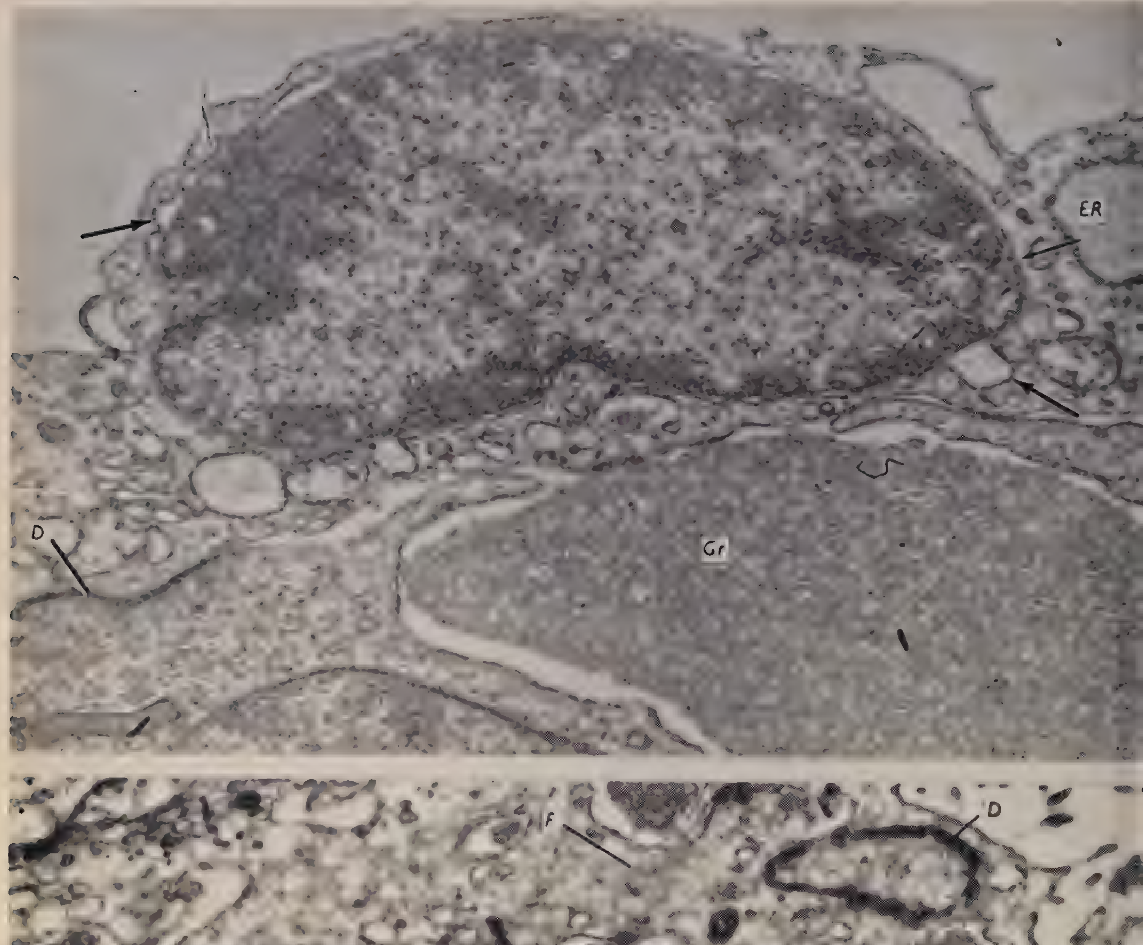

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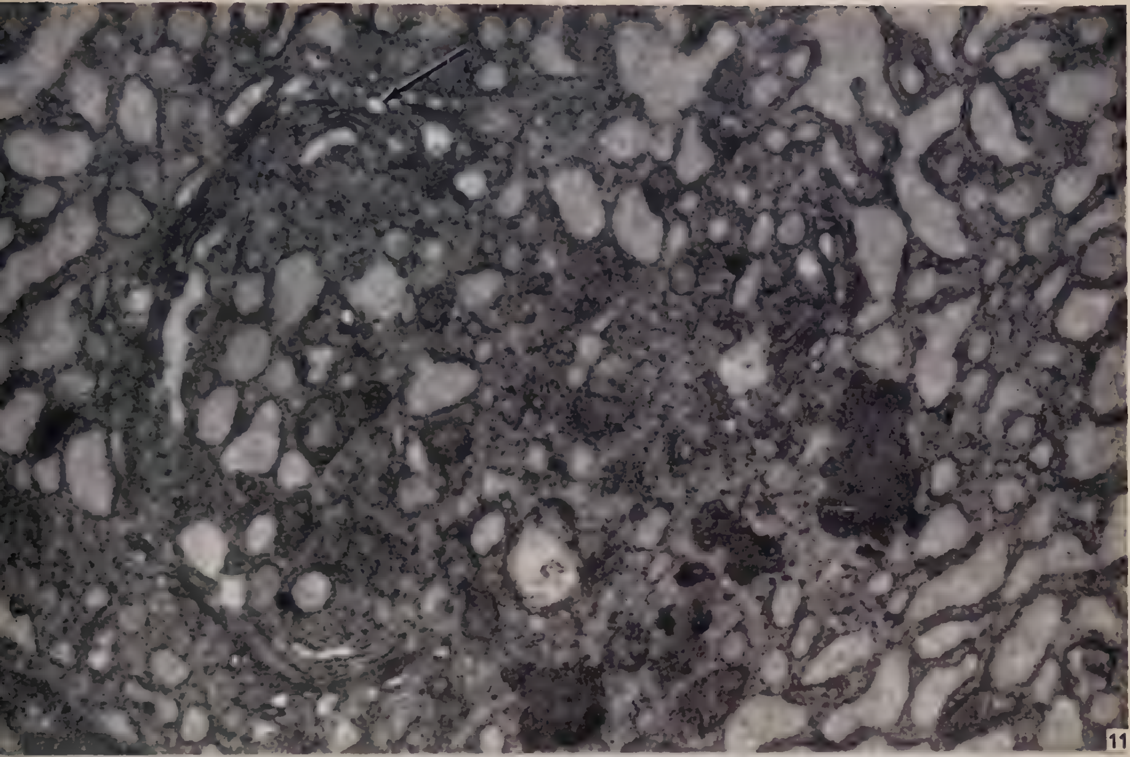

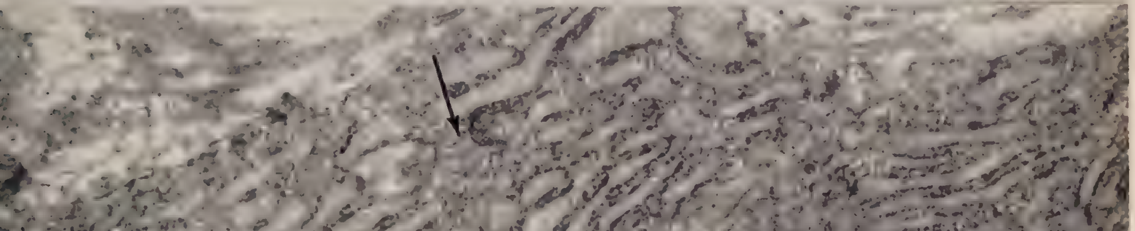

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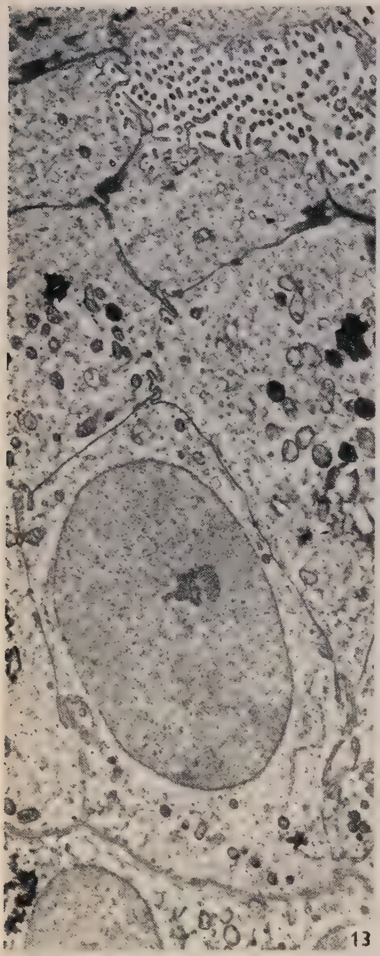
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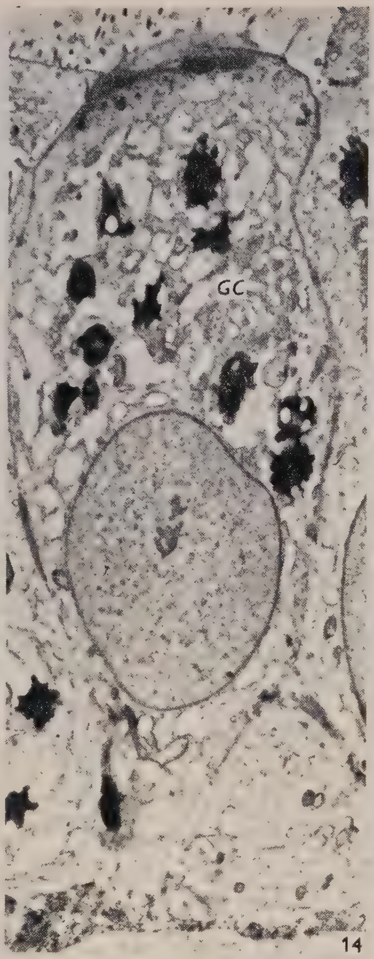

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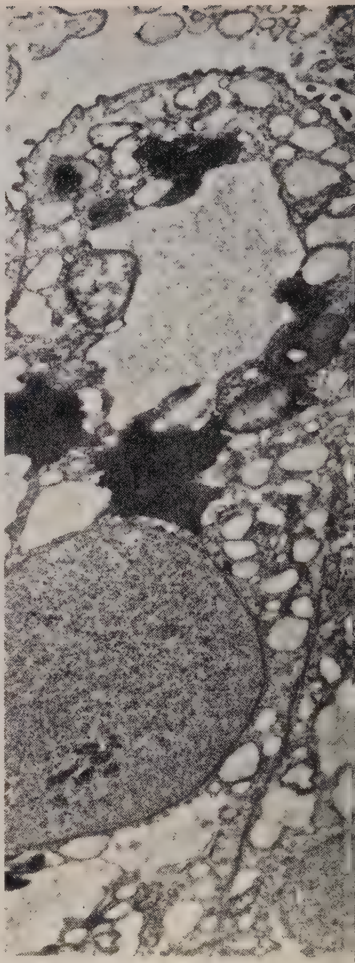

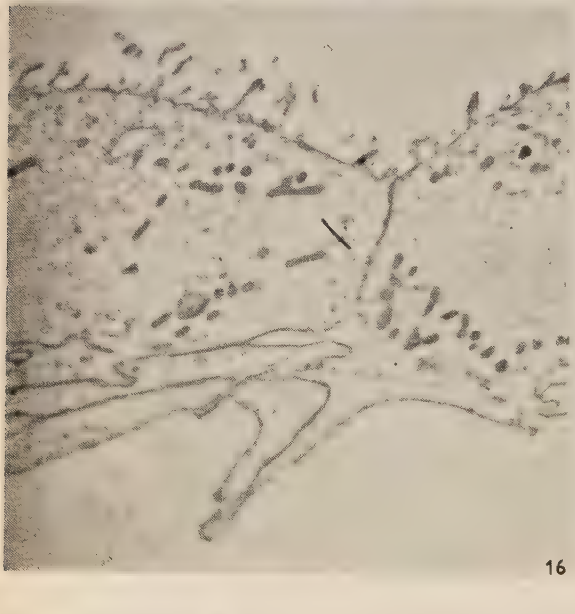
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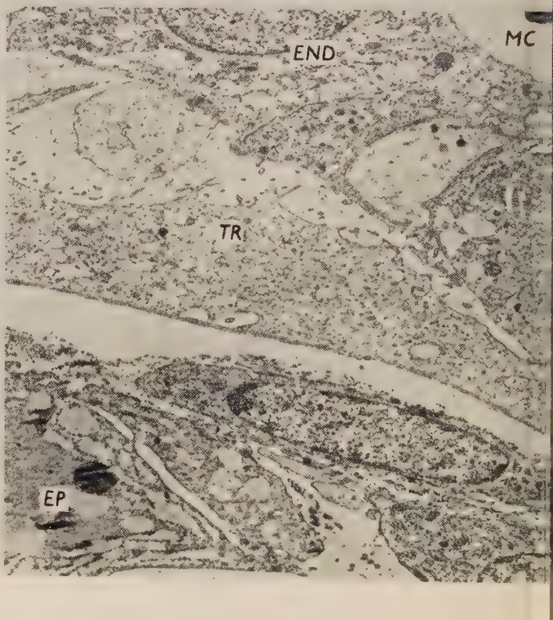


ENDERS—ELECTRON MICROSCOPIC OBSERVATIONS ON THE VILLOUS HAEMOCHORIAL PLACENTA OF THE NINE-BANDED ARMADILLO (*DASYPUS NOVEMCINCTUS*)

PLATE 2

Structure of the villi from the period of the mature placenta

Fig. 4. A marginal portion of a villus. In this particular region of syncytium the endoplasmic reticulum is in the form of vesicles, and the canaliculi are relatively collapsed. The nature of the placental barrier is clearly depicted, as is the modified fibroblast in the lower left corner. × 7600.

Fig. 5. In this section of the syncytium the canaliculi formed by the invagination of the basal plasma membrane are dilated and are especially clearly seen (arrows). × 8700.

Fig. 6. Tangential view of the surface of the syncytium. Note that the microvilli are relatively short compared with the earlier period of the placenta (fig. 3) but that they are numerous and highly branched. × 11,000.

PLATE 3

Fig. 7. Region of junction of the cell column with the vascular villus. At the upper left are processes of several mesenchymal cells. Cutting across the middle is the syncytial trophoblast. At the lower right are parts of two cells of the cell column. Note the desmosomes formed at points of contact between cells of the cell column and the syncytium (arrows) and the thinness of the basement membrane underlying the syncytium. × 7400.

Fig. 8. Typical cells of the cell column. Note the granulation of the cytoplasm (large arrows) next to the intercellular space, and that the cell membranes are frequently indistinct in these regions. Note also the numerous RNP granules in the cytoplasm (small arrow). × 18,000.

PLATE 4

Region of junction between the cytotrophoblast and the overlying syncytium

Fig. 9. Note that the outer nuclear membrane is reflected away from the nucleus at several spots, and that RNP granules are associated with these areas of the membrane (large arrows). A possible nuclear pore is seen at the small arrow. × 17,000.

Fig. 10. In this region there is considerable interdigitation between the cytotrophoblast, which is predominantly at the lower left, and the syncytium. Note that the attachment plaques of the desmosomes can be clearly distinguished, and that the intercellular granules have reached the surface of the syncytium at the right. The small arrow points to a cluster of RNP particles. × 18,700.

PLATE 5

Structure of the modified fibroblast of the mature placenta

Fig. 11. Golgi complex. Note that the Golgi vesicles (arrow) do not appear to contain the amorphous substance seen in the cisternae of the endoplasmic reticulum. × 18,000.

Fig. 12. Parts of two modified fibroblasts are seen. Of special interest is the presence of distinct granules within the cytoplasm (arrows). Note that the cisternae of the endoplasmic reticulum are oriented in relation to these areas of the cytoplasm. × 18,000.

PLATE 6

Figs. 13–15 depict the progressive modification of the glandular epithelial cells of the endometrium which occurs with the onset of implantation.

Fig. 13. Note that there are numerous microvilli and relatively few small lipid droplets in these unmodified cells. × 5900.

Fig. 14. The endoplasmic reticulum shows more extensive dilatation than in the preceding picture. Microvilli are still present on the surface. × 5500.

Fig. 15. The hypertrophy of this endometrial cell is exaggerated by the increase in magnification. Microvilli are reduced. Note the granulation within the large vesicle. In light microscope preparations these cells contain vast quantities of glycogen. × 8500.

Fig. 16. An electron micrograph of the abembryonic trophoderm of a blastocyst. Note the manner in which the cells interdigitate at their basal margins. The microvilli are on the outer surface of the blastocyst. Note also the absence of mitochondria from the more central regions of the cells, and the absence of any extracellular membranes. × 8500.

Fig. 17. A tongue of trophoblast is apparently invading between the already modified endothelium of the maternal capillary and the glandular epithelium. Note the collagen fibres and fibroblast lying beneath the trophoblast. × 5000.

FIBRE DEGENERATION IN THE CEREBRAL CORTEX OF THE CAT AND RABBIT FOLLOWING EXPERIMENTAL CRANIOTOMY

By W. G. HARRIS

Department of Anatomy, University College, London

INTRODUCTION

During the course of a series of experiments on the cats' cerebral cortex it was noted that craniotomy and exposure of the pia mater by incising the dura mater resulted in histologically demonstrable fibre degeneration in the underlying cortex. There had been no interference with the pia mater or the cortex. As experimental craniotomy is frequently performed prior to lesions being made in the cerebral cortex this observation seemed of some considerable importance. Further experiments have been carried out to verify it.

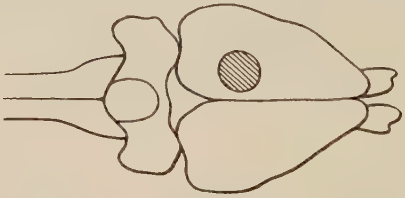
MATERIALS

Three cats and seven rabbits were used, of differing sex and breed. The cats were anaesthetized with intraperitoneal nembutal supplemented with ether when necessary. The rabbits were anaesthetized with intravenous nembutal.

METHOD

(*a*) *Rabbits*

The rabbits were divided into three pairs. In one of each pair craniotomy was performed and the dura incised, thus exposing the pia mater. In the second of each pair after craniotomy the dura was left intact. The seventh animal was the second of a pair in which the first sustained damage to the cortex during operation and has not been included in the results.



Text-fig. 1. Rabbit brain. The shaded area shows the approximate position and extent of the craniotomy.

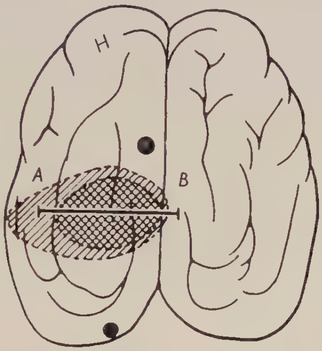
In all rabbits the craniotomy was performed in the same situation on the skull. This is shown in Text-fig. 1 in which the shaded area shows the approximate site and size of the bony defect.

The brains were perfused and removed 4 and 5 days after operation. In each animal the bony defects were measured immediately before the brain was removed.

(*b*) *Cats*

In cat 1 the bony defect was large (Text-fig. 2). The dura beneath was incised and the pia exposed. In each of the second and third cats there were two small craniotomies (Text-fig. 2) and in each of these cats the dura under only one of the defects was incised.

The brain of the first cat was perfused and removed 5 days after operation. Those of the second and third cats were perfused and removed 8 days after operation.



Text-fig. 2. Cat brain. Diagram showing: (i) Approximate position and extent of the bone defect in cat 1; shaded ▨. (ii) Approximate position and extent of the dural defect in cat 1; shaded ▩. (iii) Approximate position and extent of the bone and dural defects in cats 2 and 3; shaded ●. (iv) *AB* is the plane of the section drawn in Text-fig. 4.

(*c*) *Method of craniotomy*

(i) The head was shaved.

(ii) A mid-line incision was made from the root of the nose to the external occipital protuberance.

(iii) The occipito-frontalis muscle was incised in the mid-line and the left one retracted laterally.

(iv) The left temporal fascia was incised along its line of attachment to the skull.

(v) The left temporal muscle and periosteum on the left side of the skull were reflected laterally using a rugine.

(vi) A dental burr was used to make a hole in the skull. Care was taken not to leave the burr in contact with bone for more than four or five seconds at any one time. It was found that if it was left longer than this undue heat was generated and it was feared that this would damage the underlying cortex. When the pial vessels could be plainly seen through the bone the burr was discarded and a pair of finely pointed scissors used to pierce this last thin piece of bone and then remove it. Haemorrhage from the bone edges was controlled with bone wax. If enlargement of the skull defect was desired it was done with bone ronguers. All the rabbits and cat 1 had their skull defects enlarged with rongeurs. The defects of cats 2 and 3 were not enlarged in this way.

(vii) If the dura was to be incised it was anchored with a dural hook, raised slightly and a small cut made in it with scissors. This small cut was then enlarged in the form of a cruciate incision. It was found that the arachnoid was always incised with the dura and C.S.F. leaked from the incision as soon as it was made. The pia mater was left exposed for about 30 sec and then the dura was replaced over the surface of the cortex. No attempt was made to suture the dura.

(viii) Whether the dura had been incised or not the bone defect was covered with gelatin sponge soaked in thrombin.

(ix) Muscles were sewn back in position in one layer with interrupted thread sutures—i.e. right occipito-frontalis to left occipito-frontalis and left temporal muscles.

(x) The skin was sutured with interrupted thread. In the first cat the craniotomy performed differed from the above in that the hole through the skull was made with a trephine rather than a dental burr. The centre pin of the trephine had been removed as this had previously been found to cause damage to the underlying cerebral cortex.

(*d*) *Perfusion and section*

All animals were perfused through the aorta with normal saline followed by 10 % neutral formal-saline. After perfusion the brains were removed and placed in 10 % neutral formal-saline for 3 to 4 weeks.

$20\,\mu$ frozen sections were taken and stained by the Nauta-Gygax technique (1954). Sections were taken as follows:

Rabbits. (i) The left hemispheres of each pair of animals were sectioned and stained in pairs. The part of each hemisphere sectioned included the area of cortex under the bone defects.

(ii) Sections were taken of the right anterior cortex from the animal in which the dura had been left intact. These sections were used as controls.

Cat 1. (i) Sections taken from the cortex under the bone defect. (ii) Sections taken from the right frontal cortex and used as control.

Cats 2 *and* 3. (i) Sections taken from the cortex under the anterior and posterior defects.

(ii) Sections taken from the right frontal cortex and used as controls.

The two groups of sections taken from each animal were divided into batches. The sections in each batch were stained at the same time and in the same solutions.

RESULTS

(*a*) *Rabbits*

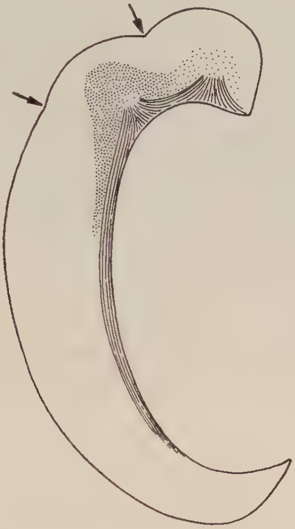
In those rabbits in which the dura had been left intact the brain appeared normal macroscopically. There was no discoloration or sign of compression and after the brain had been perfused and removed it was not possible to see which area of cortex had been underlying the bone defect.

In those rabbits in which the dura had been incised the dura had retracted to the bone edges in all the animals and the brain had herniated, to a small extent, through the dural and bone defects. When the brain had been perfused and removed a small 'hillock' was seen on its surface, with well-defined edges, clearly showing the extent of the dural and bone defects. The surface of each hillock was smooth and no haematomata were seen on any part of the herniated areas of cortex. Thus, apart from the raised area the brain appeared normal.

Size of the bone defects

These were approximately 1 cm. in diameter in all the rabbits but varied between 0·8 and 1·2 cm.

Striking signs of degeneration were seen in all the animals that had undergone craniotomy with incision of the dura, other parts of the cortex and the controls being free of degeneration (Pl. 1). The most marked degeneration was seen in the deepest parts of the cortex, but in the area under the central parts of the defect the degenerating fibres could be traced nearer to the surface. In two sections of one animal it could be traced right up to the surface. In these two sections degeneration could also be seen in the tangential fibres of layer one. As each section included the whole dorso-ventral extent of the hemisphere, cortex well away from the defect was included. In all sections this was clear of degenerating fibres (see Text-fig. 3).



Text-fig. 3. Drawing showing the extent of the degenerating fibres in a transverse section of rabbits brain. The arrows demarcate the extent of the herniation. (Drawn from a section.)

The distribution of degenerating fibres is not an indication of the actual number present. It is more difficult to stain the smaller superficial fibres with this technique and the apparent small number stained does not necessarily mean that few fibres were degenerating.

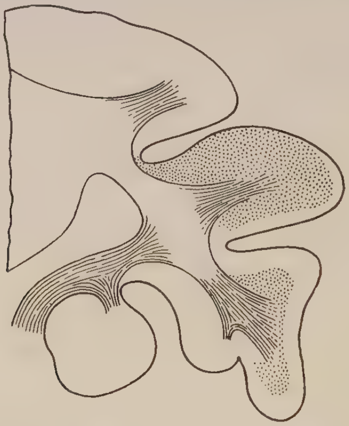
In the animals in which the dura was left intact there was either very little degeneration to be seen or none at all. Of the four animals examined three showed no degeneration and in one a small number of degenerating fibres was visible in the cortex under the defect.

(*b*) *Cats*

In all three animals the brains appeared normal. No herniation had occurred through the dural defects and after the brains had been perfused and removed it was not possible to tell which had been the exposed areas of cortex.

In *Cat* 1 the bone defect was large (4 cm. × 2·4 cm.) and after perfusing the brain it was found that the dura had not retracted right to the bone edges as in all the other animals used. It had only partially retracted and exposed an area of cortex 2·1 cm. × 1·6 cm. (Text-fig. 2). Sections were taken through the exposed area of cortex as shown in this illustration and degenerating fibres were found in great number in the area of cortex left exposed by the retracting dura (Text-fig. 4). The cortex which had been covered by dura, even though not covered by bone, was free of degeneration.

The fibre degeneration was seen through the entire thickness of the cortex, including the tangential fibres in layer one and the picture resembled that seen in the cortex following a lesion in the underlying medullary tissue (Pl. 1, figs. 1, 2).



Text-fig. 4. Cat 1. Drawing of the section indicated in Text-fig. 2 by the line *AB*. Dotted area shows the extent of the degeneration.

Cats 2 *and* 3.

Size of bone defects.

Cat 2	*Anterior*	0·6 cm. × 0·5 cm.	Dura intact.
	Posterior	0·4 cm. × 0·5 cm.	Dura incised.
Cat 3	*Anterior*	0·6 cm. × 0·4 cm.	Dura incised.
	Posterior	0·4 cm. × 0·3 cm.	Dura intact.

In both these animals the cortex under the incised dura showed degenerating fibres (Pl. 1, figs. 5, 6). In cat 2, however, its full extent could not be determined because of failure of the stain in one batch of sections. It appeared to be minimal in amount and extent. This was confirmed in cat 3. In the cortex under the craniotomy where the dura was left intact no degeneration was seen in Cat 2 but a small number of degenerating fibres were seen in cat 3.

DISCUSSION

The original suspicion that a simple craniotomy and incision of the dura will give rise to degeneration in the underlying cortex has been verified. Large cranial defects in the region of 1–3 cm. in diameter including incision of the dura result in heavy degeneration. If lesions are made in the brain through such a defect the degeneration resulting from the craniotomy will confuse the picture. However, if the bone defect and subsequent dural incision is small—in the region of half a centimetre in diameter—the resulting degeneration is minimal. It may be possible to use a simple craniotomy of over 1 cm. diameter and with incision of the dura to produce degeneration in a small area of cortex.

As it is the intention of this paper only to record the observation made, no attempt has been made to trace the exact cause and mechanism of production of the degeneration, but certain conclusions can be drawn from the known facts. When the dura was incised during craniotomy the pia was left exposed to air for about 30–45 sec before the dural flaps were placed back in position and the bone defect covered with a gelatin sponge soaked in thrombin. In these exposed areas of cortex degeneration could be demonstrated. However, in cat 1 the dura had not retracted completely to the bone edge so that the dural defect was less than the bone defect. Degeneration could only be seen in the cortex left exposed by the retracting dura. In the cortex still covered by the dura no degeneration could be seen. It seems therefore that the dura protected the cortex from whatever it was that caused the degeneration even when the dura in a neighbouring area has been damaged.

The factor causing the degeneration must be found either in the technique of the craniotomy or in the post operative period.

Damage during the craniotomy might be the cause of the degeneration. This is the main reason why a detailed description of the routine used has been given. However up to the point at which the dura was opened the procedure in all animals was the same and yet it was only in those which subsequently had the dura incised that degeneration regularly occurred. This exonerates from blame all the steps before this stage, including the use of rongeurs for enlarging the bone defect. The use of this instrument with its thick beaks almost certainly resulted in some pressure being applied to the cortex but degeneration did not necessarily result from this. Similarly, the use of the dural hook to anchor the dura before its incision almost certainly resulted in no harm to the cortex even though several attempts have to be made to ‘catch’ the dura. It seems, therefore, that the cause of degeneration must be sought in the post-operative period when some factor was acting on the unprotected area of cortex.

In no animal or section examined was any necrosis of brain tissue seen. There was no mass destruction of tissue and so no direct interference with nerve fibres. This would indicate that any mechanical pressure applied to the cortex was not severe and certainly not sufficient of itself, to cause fibre degeneration. That no pressure was in fact exerted can be seen from the fact that herniation of the rabbit brains occurred when the opposite effect would be expected if a pressure had been applied to the cortex. For degeneration to occur in the depths of the cortex as seen in the rabbits it is probable therefore that the factor responsible acted on the pial vessels, not on the cortex itself and produced a vascular spasm. This spasm resulted in ischaemia of the underlying cortex of sufficient severity to cause the degeneration seen, but insufficient to cause necrosis.

Shortly after opening the dura the author has often seen spasm of the pial vessels, without any stimulation being directly applied to them. Echlin in 1942 showed that constriction of the pial arteries of the cat occurred after electrical or mechanical stimulation. Following this he was also able to show that a focal cerebral ischaemia resulted and that the spasm and resulting ischaemia remained localized to the area stimulated. The mechanical stimulation used by Echlin was stretching or stroking the vessel wall and it seems that the type of stimulation to which the vessels in the exposed areas of pia following the craniotomies were exposed could have been similar. Further from Echlin's experiments it can be argued that a vascular spasm was set up which was localized in extent and gave rise to a local area of ischaemia.

Holmes, Highet & Seddon (1944), in their studies of peripheral nerve changes in Volkmann's contracture, showed that it is possible to have a degree of anoxia sufficient to cause Wallerian degeneration in a nerve without necrosis. There is no reason to suppose, therefore, that a degree of anoxia may not be brought about in the cortex sufficient to cause fibre degeneration but insufficient to cause necrosis.

Apart from the mechanical factors causing a vascular spasm there is also the effect of gelatin sponge and thrombin on the cortex to be considered. Gelatin sponge soaked in thrombin was always applied to the bone defects before the operation was completed and in those animals in which the pia was exposed these substances would be in direct contact with it. As a result they may themselves cause a vascular spasm similar to that described above or have a direct action on the cortex.

Whatever the factor may be which causes this degeneration a large bone and dural defect will give rise to degeneration in the underlying cortex of such quantity that it may be difficult or impossible to differentiate it from that caused by a subsequent lesion in or under the cortex. On the other hand, a small bone and dural defect gives rise to minimal degeneration and if the dura is left intact there may be no degeneration at all.

SUMMARY

1. Experimental craniotomy in rabbits and cats with incision of the dura will result in fibre degeneration in the underlying cortex.

2. Small bone and dural defects, in the region of 0·5 cm. in diameter, will result in minimal degeneration.

3. Large bone and dural defects (over 1 cm. in diameter) will result in profuse degeneration.

4. Experimental craniotomy leaving the dura intact does not necessarily result in fibre degeneration.

I wish to thank Prof. J. Z. Young for his continuous support and help during these experiments and in the subsequent written account. Also Dr D. Sholl for his encouragement and suggestions. In addition I would like to thank Mr Armstrong for the photomicrographs, Mr Lee for the drawings and Miss Shira for technical assistance.

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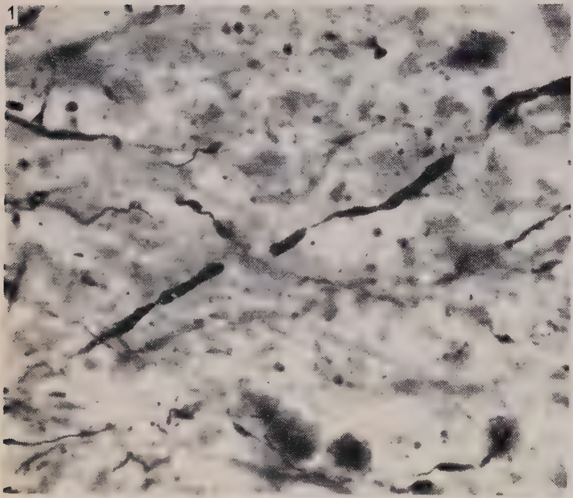
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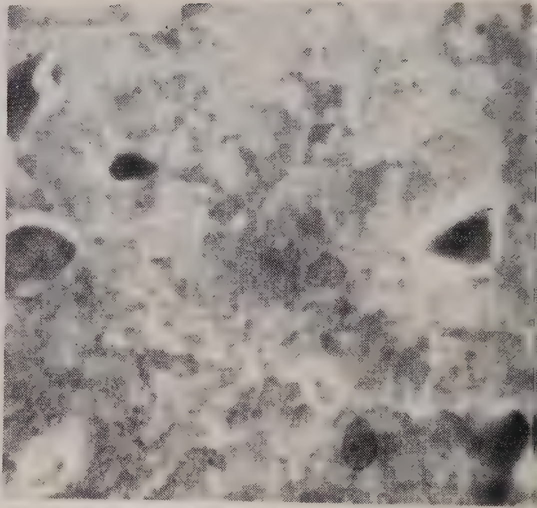

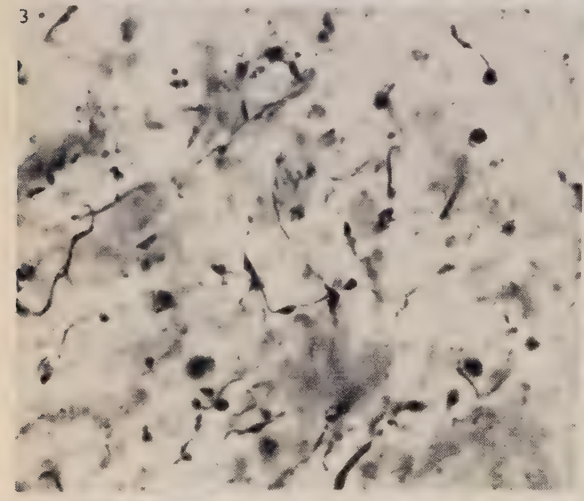
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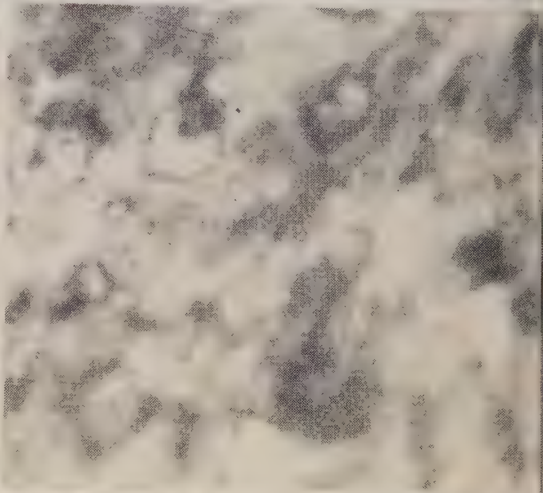

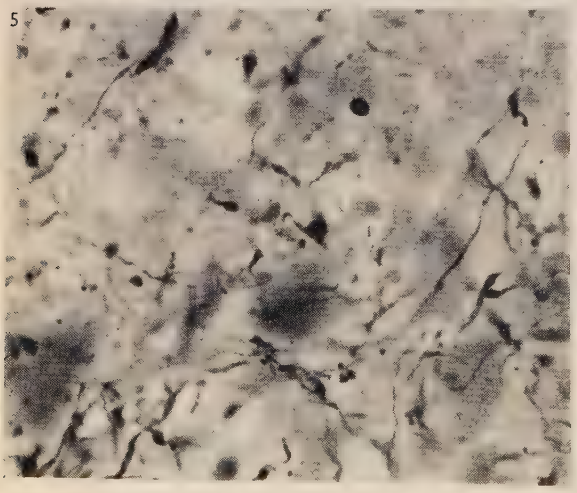
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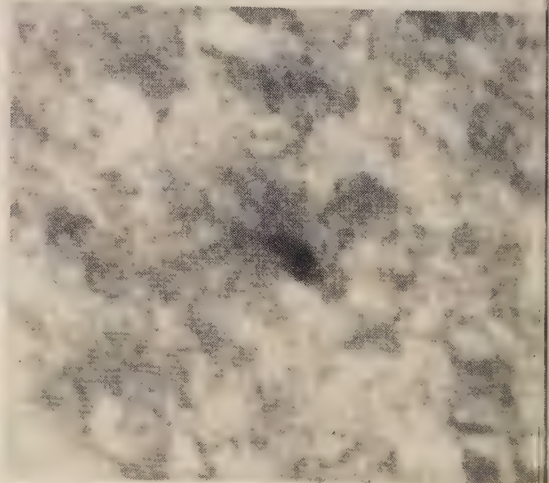

HARRIS —Fibre degeneration following craniotomy

EXPLANATION OF PLATE

(All × 1100.)

Figs. 1, 2. Cat 1. Fig. 1. The section shows a large degenerating fibre in the cortex underlying the dural defect. Fig. 2. Control section from the right frontal region.

Figs. 3, 4. Rabbit 1. Fig. 3. Degenerating fibres and terminals in the exposed cortex. Fig. 4. This is the same section as Fig. 3 but shows an area of cortex which had not been exposed.

Figs. 5, 6. Cat 2. Fig. 5. A section showing degenerating fibres and terminals in the exposed cortex. Fig. 6. Control from the right frontal region.

SOME OBSERVATIONS ON DIAPHRAGMATIC BLOOD SUPPLY

By F. BECK and J. S. BAXTER

Department of Anatomy, University College, Cardiff

Much detailed work has been done on the nerve supply of the diaphragm in man and animals, and the results obtained have resulted in important conclusions concerning the development of the diaphragmatic musculature. The blood supply of the diaphragm in mammals is less well known, and indeed Green (1955) in her monograph on the anatomy of that commonly used laboratory animal, the rat, gives little detail of the arterial supply and does not mention the venous drainage at all. We have studied the vascular pattern of the diaphragm, chiefly in the rat, preliminary to an investigation of its development.

MATERIAL AND METHODS

Preparations of the blood vessels of the diaphragm were made in twenty-eight adult and four new-born rats in several ways. (1) Coloured neoprene latex was injected into either the arterial or venous system or in some cases into both. In two new-born rats indian ink was the injection medium. (2) Intravascular haemoglobin was stained by Pickworth's method (as modified by Pfaff & Williams, 1940) in four adult specimens. Blood vessels were stained by Grant's (1929) technique in three adults. We found the latter method gave good results when certain modifications were made, namely, the injection of acetylcholine and sodium citrate into the circulation immediately on death of the animal and continuance of the perfusion, after this, with well-filtered Ehrlich's haematoxylin in 10% formalin at a pressure of 120 mm. of mercury for 3 hr. (3) Histological sections at various key areas of the diaphragm in five adult rats were stained with haematoxylin and eosin and by van Gieson's method.

We have also made injection preparations of the diaphragm from an adult dog, an adult cat and two adult specimens of the fruit bat (Pteropus).

RESULTS

The vascular pattern of the diaphragm in the rat

(*A*) The arterial supply of the rat's diaphragm is shown in Plate 1, fig. 1, in which the preparation is viewed from the abdominal aspect.

The arterial vessels in the rat diaphragm are these:

(1) Inferior phrenic vessels (left and right).
(2) Superior phrenic or pericardiaco-phrenic vessels.
(3) Intercostal rami.
(4) Musculo-phrenic arteries.
(5) Twigs from the internal mammary arteries.

There are some further points about these vessels which should be mentioned.

(*a*) The inferior phrenics, arising from the abdominal aorta, are the chief supply of the crura and the central area of the pars costalis; they are often supplemented by accessory inferior phrenic arteries.

(*b*) The peripheral part of the muscular diaphragm is vascularized by twigs from the 7th to 12th posterior intercostal arteries, by radicles from the loop anastomoses between the 6th anterior and 6th posterior intercostal arteries (the musculo-phrenic artery) and by terminal branches of the internal mammary arteries.

(*c*) The superior phrenic or pericardiaco-phrenic arteries are small vessels accompanying their respective phrenic nerves to the diaphragm and are of small importance.

(*B*) The venous drainage of the diaphragm presents a remarkably constant pattern which is shown in Plate 1, fig. 2, the position of these veins being of significance when considered in terms of the function of this structure.

The venous drainage is mediated by four main channels on each side. They are:

(1) Anterior phrenic vein.
(2) Middle phrenic vein.
(3) Posterior phrenic vein.
(4) Accessory posterior phrenic vein (a composite venous channel).

Small diaphragmatic tributaries of the musculo-phrenic and intercostal veins drain the periphery of the diaphragm and anastomose with tributaries of the anterior middle and posterior phrenic veins (Pl. 2, fig. 1). A cross-anastomosis between the veins of the right and left sides of the diaphragm called the transverse phrenic vein completes the symmetrical pattern.

Certain features of these veins have now to be detailed.

(*a*) The anterior phrenic veins run antero-posteriorly at the boundary between the pars costalis and the anterior leaf of the central tendon (Pl. 2, fig. 3). Straight radicles pass from the muscular part of the diaphragm into the anterior phrenic veins. These are vessels which are parallel to the longitudinal direction of the muscle fibres and drain the pars costalis. Peripherally they anastomose with the lower intercostal, internal mammary and musculo-phrenic veins. The right anterior phrenic vein terminates, as a rule, in the middle phrenic vein near the caval orifice and thus forms an arc around a part of the circumference of this opening. The left anterior phrenic vein ends in the transverse phrenic vein within the boundary of the central tendon. The very anterior part of the anterior phrenic vein is occasionally found to run within the muscle of pars costalis for a short distance before it reaches the boundary between muscle and tendon. In these cases another vein joining the anterior phrenic vein where the latter reaches the boundary is found between the muscle and tendon further anteriorly (Pl. 2, fig. 2).

(*b*) Middle phrenic veins. The right vein opens directly into the inferior vena cava as this passes through the diaphragm, while the left joins the posterior phrenic vein at the junction of muscle and central tendon to form the transverse phrenic vein. The middle phrenic veins again run parallel to the long axis of the muscle fibres and their tributaries show a ‘stepped’ appearance as if made up of centripetally running vessels connected by cross-anastomoses (Pl. 2, fig. 2). They are responsible for the drainage of a large part of the pars costalis and have peripheral connexions with the 7th to 9th intercostal veins.

(*c*) Posterior phrenic veins. The termination of the right vein is in the inferior vena cava while the left uniting with the left middle phrenic vein forms the transverse phrenic vein. Traced backwards, each vessel runs just within the boundary of the central tendon to the apex of the dorso-lateral leaf and then lateral to the lumbo-costal trigone. Each vein receives numerous straight tributaries on the lateral side parallel to the muscle fibres and these connect peripherally with the lower intercostal veins. There are some medial tributaries, the most important being two on the right side (Plate 1, fig. 2) related to the right inferior phrenic artery and coming from the pars lumbalis of the diaphragm. They communicate with the right accessory posterior phrenic vein.

(*d*) Accessory posterior phrenic veins. These veins arise by a number of small radicles in the crura where they have anastomoses with the azygos system and the 1st lumbar veins. They then run towards and cross the dorso-lateral leaf of the central tendon, first communicating with each other along its medial margin and ending, after having traversed the tendon, in the inferior vena cava, the right posterior phrenic vein, the transverse phrenic vein and the left posterior phrenic vein. They thus form a venous cross-connexion in the medial margin of the dorso-lateral leaf of the central tendon—the accessory posterior phrenic vein.

(*e*) Transverse phrenic vein. This is an important large vein connecting the left side of the diaphragmatic venous drainage with the inferior vena cava. It receives all the major veins of the left half of the diaphragm and begins at the boundary between the central and left dorso-lateral leaves of the central tendon. It passes to the right just within the central tendon near to the two fused crura. The vein has a few small tributaries from the anterior leaf of the central tendon and others which have already been mentioned.

(*f*) Diaphragmatic tributaries of the musculo-phrenic and intercostal veins were clearly demonstrated in animals where the azygos system had been injected, and also in preparations of the blood vessels made by Grant's method (Pl. 2, fig. 1). They drain the peripheral part of the diaphragm and anastomose in series with the anterior phrenic, middle phrenic and posterior phrenic veins (Pl. 2, fig. 1).

Small veins draining the crura appear to run to the lumbar veins or to the azygos vein as this pierces the diaphragm.

The superior phrenic vein (Pl. 2, fig. 1) running with the phrenic nerve has a small and inconstant area of drainage.

Brief additional notes on the diaphragm of the bat, cat and dog

The arterial supply in the bat is very similar to that found in the rat except that the inferior phrenic arteries arise by a common stem from the abdominal aorta (Pl. 3, fig. 1). The venous drainage again is remarkably like that in the rat with the familiar pattern of anterior, middle, posterior and accessory posterior phrenic veins 'framing' the central tendon on each side and connected by the transverse phrenic vein (Pl. 3, fig. 2). The commencement of both posterior phrenic veins and a part of the right anterior phrenic vein run within the muscle at right angles to its fibres, but this does not appear to be a significant difference, since the muscle on the central tendon side of the veins is much attenuated in comparison with the main portion of the pars costalis on their lateral side.

The diaphragmatic venous drainage in the dog (Pl. 3, fig. 3) shows the same pattern as in the rat. Where the beginning of both anterior phrenic veins appears to be embedded in muscle and the course to be at right angles to the direction of the muscle fibres the thickness of the diaphragmatic muscle on the medial and lateral sides of the vein is precisely the same as in the bat. Furthermore, a small vein is found on the left side at the junction of muscle and tendon in addition to the main anterior phrenic vein when the latter is running in the muscle.

The veins of the diaphragm in the cat (Pl. 3, fig. 4) show, as it were, a standard pattern, except that the dorso-lateral leaf of the central tendon is very narrow and accessory posterior phrenic veins are absent.

DISCUSSION

The foregoing, strictly morphological, account of the vascular supply of the diaphragm provides a necessary basis for further work in this field. From it the following points emerge.

(1) The venous drainage of the diaphragm is very constant in all the specimens examined, and furthermore a remarkable bilateral symmetry is immediately apparent. The diaphragmatic veins are the only tributaries of that component of the adult inferior vena cava which is developed from the hepatocardiac channel and this segment is itself the right member of the originally bilateral vitelline veins (Streeter, 1942).

The problem now arises as to whether the drainage of the left half of the diaphragm is into tributaries of the left vitelline vein which has become secondarily connected with the right vitelline vein by means of the transverse phrenic vein; in the same way, for example, that the intersubcardinal anastomosis connecting the two primitive subcardinal veins, persists in the adult as the left renal vein and allows the cephalic part of the left subcardinal vein to regress as a consequence.

Blair (1922) described in the human a perforating vein present fairly constantly at a point on the left side corresponding to the position of the caval orifice. This he considered to represent a continuation of the left vitelline vein above the diaphragm. The perforating vein he described eventually joined the inferior vena cava by means of a transverse vein running on the superior surface of the diaphragm. No evidence of such perforating or transverse vein was seen macroscopically in the specimens studied here. Of course this finding does not exclude its occasional presence but even if it were never present, the transverse phrenic vein could still represent the foetal connecting channel between the right and left vitelline veins running on the abdominal surface of the diaphragm.

Work is at present being done by injection methods on rat foetuses to elucidate the origin of the diaphragmatic veins.

(2) The second point of interest concerning the venous drainage of the diaphragm is the constant position of the main venous channels just within the central tendon of the diaphragm. These veins run at right angles to the direction taken by the diaphragmatic muscle fibres, they lie on the abdominal surface of the diaphragm and

their centrifugal tributaries run parallel to the direction of the muscle fibres and are buried within the substance of the muscle.

Clark (1918) enlarging on the work of Thoma (1893) dealt with the development of blood vessels in tadpoles; he considered that a certain degree of development of blood vessels is genetically determined but that beyond a certain point mechanical and functional factors cause the final pattern. Two of these factors are that:

(i) The amount of blood flow through a vessel regulates its diameter.

(ii) Increase in the length of a vessel is due to surrounding tissue tension.

Anrep (1934 *a*, *b*) has studied the effect of muscular contraction upon blood flow and concludes that the blood flow through the diaphragm is diminished during its normal spontaneous contractions. This diminution in the amount of blood flow presumably affects the muscular veins most severely, particularly those which run across the direction of the muscle fibres. It therefore seems reasonable to assume that such transversely (in relation to the muscle fibres) running veins would not develop in the embryo from the pre-existing capillaries—which form a network from which the adult arteries and veins will differentiate—until the region of the central tendon is reached where muscular contraction cannot further affect blood flow through the veins. This is found to be the case in the diaphragms studied. One might consider its part in venous drainage to be one of the functions of the central tendon. Furthermore, the second of Clark's mechanical factors quoted above would ensure that the centrifugal tributaries of the central veins would run parallel to the direction of the muscle fibres, as indeed they do.

Following this line of argument it is unnecessary to postulate the existence of a left vitelline venous system to account for the symmetry of the diaphragmatic venous drainage; nevertheless, such a basic genetic system is not disproved by the finding that reinforcing mechanical factors exist. In fact the existence of some veins running in the muscle and yet transversely to the long axis of the muscle fibres, such as the anterior part of the anterior phrenic veins in the dog, suggests an hereditary basis since mechanical factors cannot have operated in the production of these particular vessels.

(3) The specimens studied clearly demonstrate an anastomosis between the caval and azygos venous systems involving the diaphragmatic veins. The normal and potential functioning of this anastomosis was not investigated, though its existence in the cat has previously been stated by Sudzilovskii (1958).

(4) As a corollary to the above it is of interest to note that blood enters the pars costalis from the periphery and leaves it centrally, in other words the common arrangement of arteries entering and veins leaving a muscle at the same hilum does not exist here. The relationship of this arrangement to muscular contraction may be worthy of investigation.

(5) Belou (1934) in his work 'Revision anatomica del sistema arterial' shows an arteriograph of the arterial supply of the human diaphragm. The similarity with the above descriptions is striking.

SUMMARY

The distribution of the arteries and veins in the diaphragm of the rat has been studied by: (1) injection methods; (2) the staining of gross specimens (methods of Pickworth and Grant) and (3) the examination of microscopic sections. Comparison is made with the vessels in the fruit bat (Pteropus), the cat and the dog.

The arterial supply is derived from: (1) inferior phrenic vessels; (2) superior phrenic or pericardiaco-phrenic vessels; (3) intercostal vessels; (4) musculo-phrenic vessels; and (5) internal mammary vessels. The courses of these vessels and the areas supplied are described.

The venous drainage by anterior, middle, posterior and accessory posterior phrenic veins is found to be remarkably constant in its main features. The major veins lie just within the confines of the central tendon and show a bilateral symmetry. They represent the only tributaries of the embryonic hepato-cardiac channel (Streeter, 1942) to be found in the adult. Two hypotheses, one genetic and the other mechanical, regarding the formation of these veins are advanced and discussed.

The presence of an anastomatic system of diaphragmatic veins connecting the inferior vena cava and azygos veins first noted in the cat by Sudzilovskii is confirmed in the rat.

We should like to thank Messrs A. Welch and L. Jones for taking the photographs for us and Miss M. White for her painstaking production of the illustrations.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Arterial supply to the diaphragm of the rat. *a*, accessory right inferior phrenic artery; *b*, 12th rib; *c*, right inferior phrenic artery; *d*, its oesophageal branch; *e*, branches of posterior intercostal artery; *f*, caval orifice; *g*, branches of musculo-phrenic artery; *h*, branches of internal mammary artery; *i*, oesophageal orifice; *j*, oesophageal branches of left inferior phrenic artery; *k*, origin of lateral branch; *l*, left inferior phrenic artery; *m*, aortic orifice.

Fig. 2. Venous drainage of the diaphragm of the rat (viewed from the abdominal aspect). *a*, lumbo-costal trigone; *b*, caval orifice; *c*, transverse phrenic vein; *d*, left anterior phrenic vein; *e*, left middle phrenic vein; *f*, left posterior phrenic vein; *g*, right and left accessory posterior phrenic veins.

Plate 2

Fig. 1. Rat diaphragm stained by Grant's method demonstrating the anastomoses between the azgyos and caval venous systems. Note the left superior phrenic vein indicated by arrow. $\times 2\frac{1}{4}$.

Fig. 2. Rat diaphragm with the veins injected with neoprene latex. Note the anterior part of the right anterior phrenic vein running within the pars muscularis, a smaller tributary indicated by an arrow marks the boundary between muscle and central tendon in this region. $\times 2\frac{1}{4}$.

Fig. 3. Section through the right anterior phrenic vein to show its relation to the muscle (*M*) and central tendon (*C.T.*) of the diaphragm. The vein (*V*) (filled with neoprene) lies on the thoracic surface of the diaphragm at this point and is overlapped on the abdominal surface by a tongue-like process of tendon which is continuous with the looser connective tissue of the central tendon. ×100.

Plate 3

Fig. 1. Diaphragm of fruit bat injected with neoprene latex to display inferior phrenic arteries. Note division of common trunk (at arrow) giving rise to right and left inferior phrenic arteries which in their further course accompany branches of the middle phrenic vein. $\times \frac{7}{5}$.

Fig. 2. Diaphragm of fruit bat injected with neoprene into arterial and venous systems. Note similarity of venous drainage to that described in rat (see text). $\times \frac{3}{2}$.

Fig. 3. Diaphragm of dog injected with neoprene to show venous system. Note similarity of venous drainage to that of rat, cat and bat (see text). A small vein (*a*) marks the boundary between the anterior leaf of the central tendon and the muscle beyond the point where the main stem of the left anterior phrenic vein can be traced peripherally into muscle. The vessel seen medial to this (*b*), within the central tendon, is an artery. $\times \frac{2}{5}$.

Fig. 4. Diaphragm of a cat injected with neoprene latex to display the diaphragmatic veins. The anterior and posterior phrenic veins lying just within the central tendon are clearly seen. Here the posterior leaves of the central tendon are narrow and consequently there is no accessory posterior phrenic vein. $\times \frac{2}{3}$.

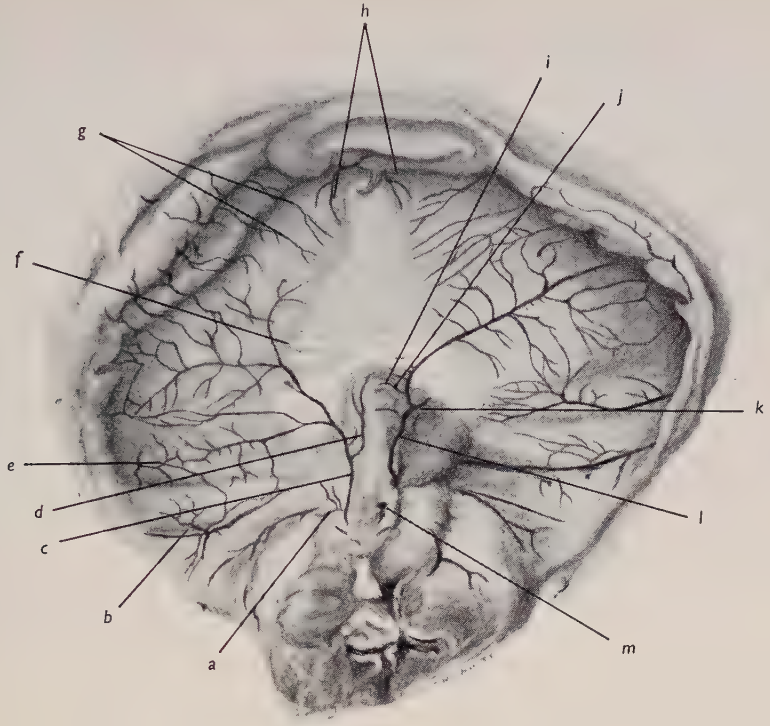


Fig. 1

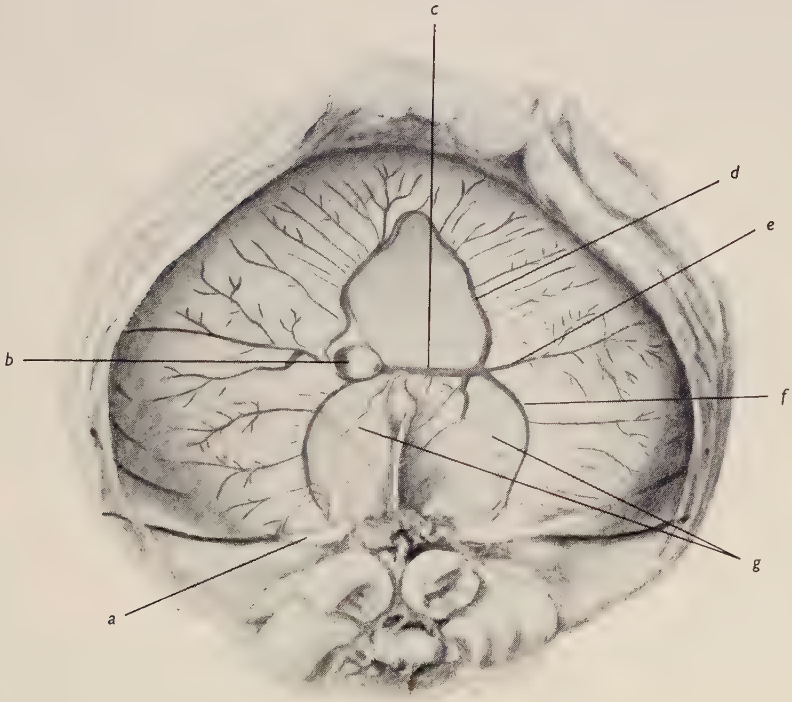
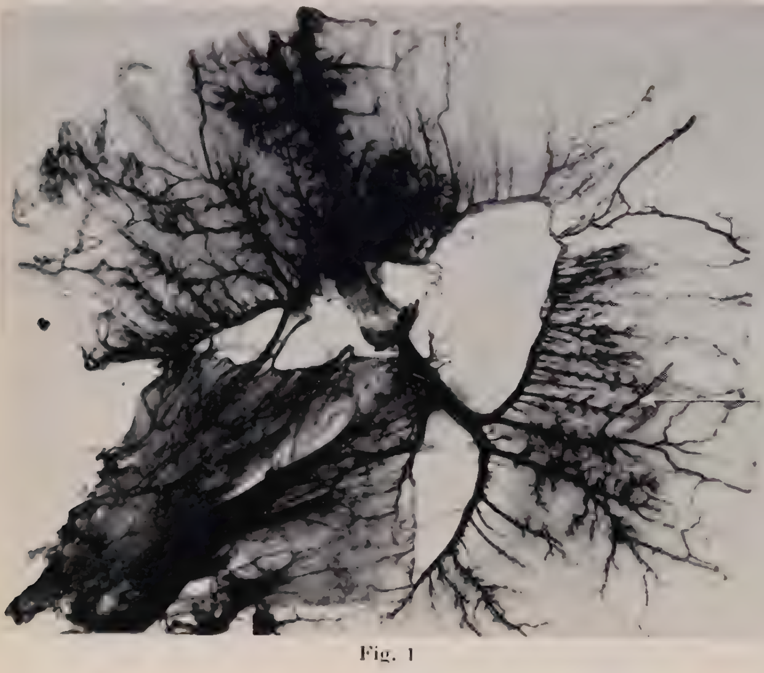


Fig. 2

BECK & BAXTER—Some observations on diaphragmatic blood supply

(*Facing p.* 230)



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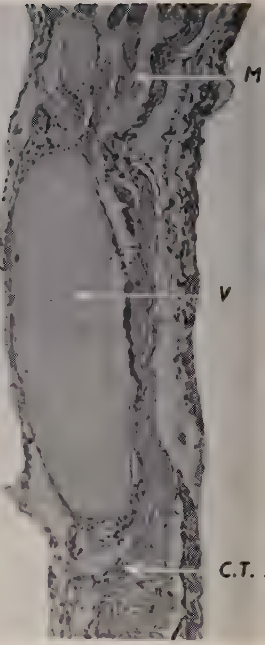


Fig. 3

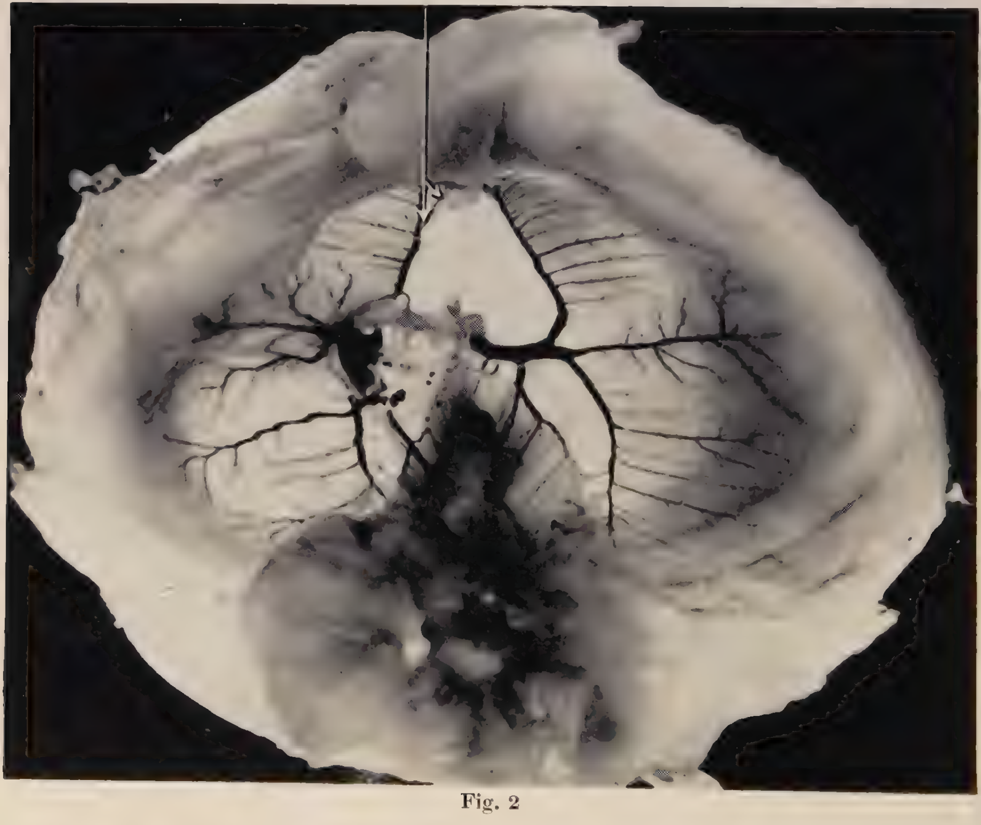
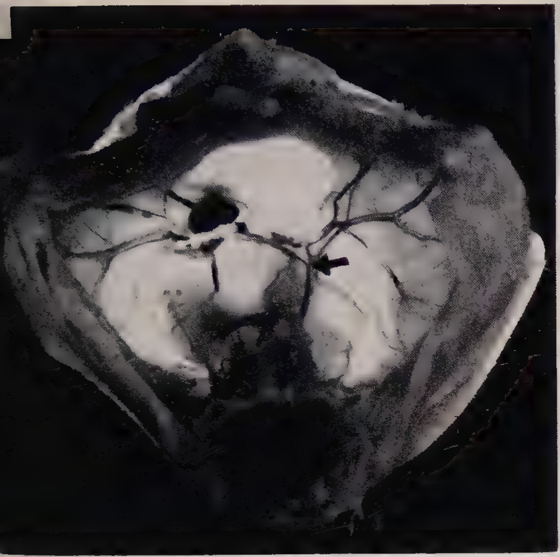


Fig. 2

BECK AND BAXTER—SOME OBSERVATIONS ON DIAPHRAGMATIC BLOOD SUPPLY



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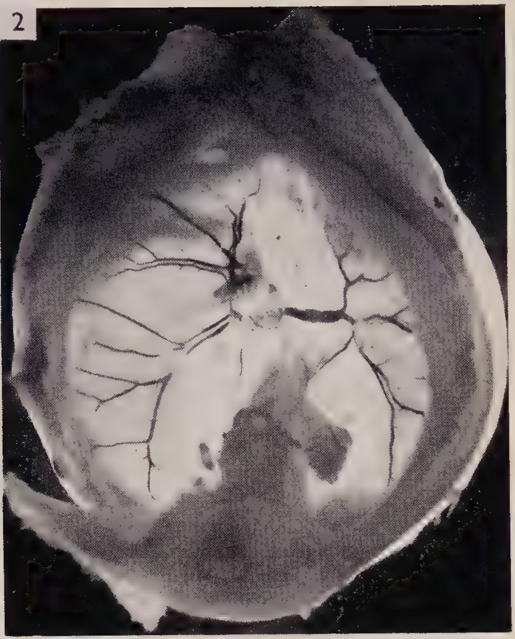

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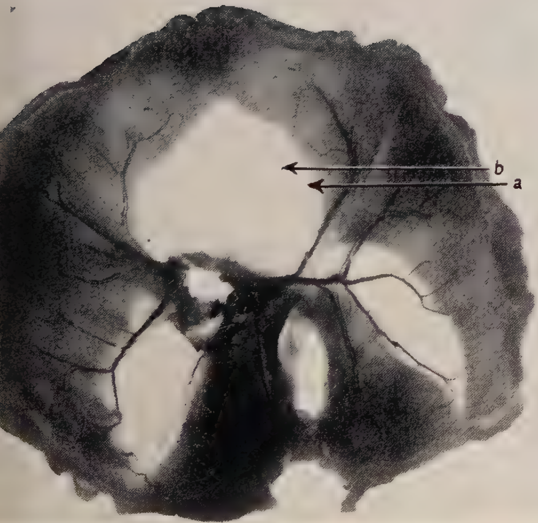

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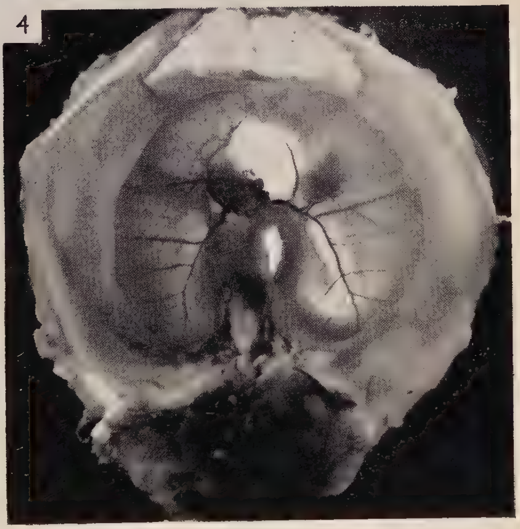

BECK AND BAXTER—SOME OBSERVATIONS ON THE DIAPHRAGMATIC BLOOD SUPPLY

SOME OBSERVATIONS ON THE DEVELOPMENT OF THE HUMAN DUODENUM

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Existing accounts of the development of the duodenum (Tandler, 1900; Lewis, 1912; Frazer & Robbins, 1915; Frazer, 1919; Pernkopf, 1925) leave much unexplained, and current text-books of embryology (Jordan & Kindred, 1936; Patten, 1946; Keith, 1948; Arey, 1949; Hamilton, Boyd & Mossman 1952), give only incomplete accounts. The remarkable overgrowth of the epithelium which obliterates the lumen of the duodenum, the migration of the biliary opening from a ventral position to its definitive site on the medial wall of the duodenum and the modifications in the ventral mesentery are developmental adaptations which have not been adequately explained. Again, several theories have been advanced to account for the 'rotation' of the midgut loop and for its return into the abdomen which occurs consistently in embryos of about 40·0 mm. crown-rump length. The present study is an attempt to clarify some of these problems and to correlate them with normal developmental changes occurring in the duodenum.

MATERIAL AND METHODS

Fourteen serially sectioned human embryos, ranging from 3·8 to 99·0 mm. crown-rump length, were examined and graphic reconstructions of all embryos up to the 37·0 mm. stage were made. For purposes of convenience, the embryos are classified into three age groups (Table 1) as follows:

Group I: embryos of 3·8—9·5 mm. C.R. length. The caudal duodenum occupies the cranial part of the midgut mesentery or lies along the right face of this mesentery.

Group II: embryos of 14·0—60·0 mm. C.R. length in which the horizontal part of the duodenum is formed.

Group III: embryos of 78·0—99·0 mm. C.R. length in which the duodenum shows an ascending part.

Table 1. *Embryos examined*

		mm.	μ
Group I	H 86	3·8	10
	H 710	4·0	4
	H 563	6·0	5
	H 177	7·75	10
	H 67	9·5	8
Group II	H 241	14·0	8
	H 242	18·0	10
	H 211	26·0	10
	H 549	37·0	7
	H 612	45·0	6
	H 653	46·0	7
	H 183	60·0	10
Group III	H 657	78·0	7
	H 80	99·0	15

All of the embryos were sectioned in the transverse plane.

In this communication the term 'embryo' is used in a broad sense and includes specimens which have crown-rump lengths of over 30·0 mm.

OBSERVATIONS

Group I. 3·8—9·5 *mm. crown-rump length embryos*

The 3·8 mm. embryo is the smallest of the group but, in fact, is more advanced in development than the 4·0 mm. embryo. Both, however, belong to horizon 13 of Streeter (1945).

In all embryos of this group the caudal duodenum passes to the right of the midline. In the 6 mm. embryo, the caudal duodenum continues as the cranial limb of the midgut loop. In the 7·75 and 9·5 mm. embryos almost the entire duodenum passes dorsally and towards the right, forming the cranial limb of a U-shaped loop whose caudal limb is formed by the proximal part of the jejunum. It should be here stated that, in embryos of this group, the proximal and distal ends of the duodenum are not clearly definable from the distal part of the stomach and from the proximal jejunum, respectively. The lumen of the duodenum is a dorso-ventral slit in the younger embryos but becomes more rounded in the 6·0 and 7·75 mm. embryos. In the 9·5 mm. embryo the lumen is flattened in the transverse plane.

The epithelium of the 4·0 mm. embryo consists of two or three layers of columnar cells between which a few cuboidal cells are interposed. The duodenal epithelium in the 3·8 mm. embryo shows commencing proliferation along its right wall, close to the origin of the hepatic diverticulum. In the 6·0 mm. embryo the epithelium is similar to that of the 3·8 mm. embryo, but in the 7·75 mm. embryo the proliferated epithelium blocks the right half or more of the duodenal lumen. This proliferation is even more pronounced in the 9·5 mm. embryo and is seen both proximal and distal to the biliary opening.

In the 3·8 and 4·0 mm. embryos the hepatic diverticulum, which represents the future bile duct, arises from the ventral aspect of the duodenum. The dorsal pancreatic bud is represented by a clump of cells in the dorsal wall of the duodenum in the 4·0 mm. embryo but, in the 3·8 mm. embryo, it is easily recognized as a single vesicle with a central lumen (Pl. 1, fig. 1). The lumen of the dorsal pancreatic bud does not, at this stage, communicate with the cavity of the duodenum. In the 6·0 mm. embryo the cavity within the dorsal bud has become so enlarged that it appears to be the direct continuation of the duodenal lumen. The ventral pancreatic bud, composed of a solitary vesicle, arises from the right and ventral part of the bile duct (Pl. 1, fig. 2). The biliary opening of the 7·75 mm. embryo is situated on the dorsal part of the right wall of the duodenum, while the dorsal pancreatic duct opens into the dorsal part of the left duodenal wall. In all embryos of this group, with the exception of the 9·5 mm. embryo, the dorsal pancreatic duct opens more cranially into the duodenum. In the 9·5 mm. embryo the two ducts open at the same level (Pl. 1, fig. 3). This embryo is the first of the series to show prolapse of the midgut loop into the umbilical coelom and the original cranial limb has now become the right limb of the loop. The right lobe of the liver is very much larger than the left lobe and almost comes into contact with the structures of the posterior abdominal wall.

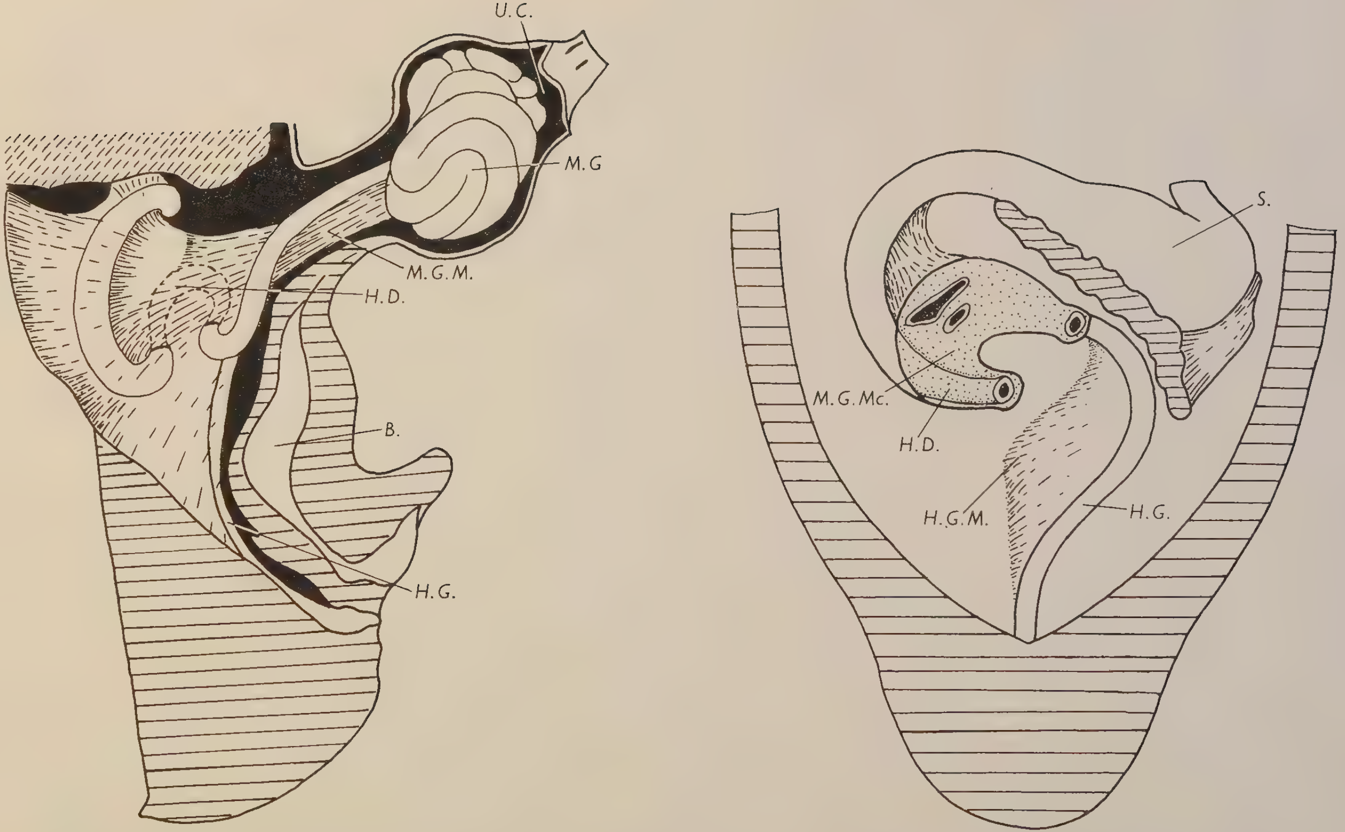
In the 3·8 and 4·0 mm. embryos the dorsal mesentery is short and thick and lies along the median plane. It is more elongated in the dorso-ventral plane in the 6·0 mm. embryo. At later stages (7·75 and 9·5 mm.) the caudal part of the duodenum lies close to the right surface of the dorsal mesoduodenum which contains the large vitelline vein. This vein, in the 7·75 mm. embryo, has a lumen 4–5 times that of the duodenum.

The ventral mesentery of the 3·8 mm. (Pl. 1, fig. 1) and 4·0 mm. embryos is broadly attached to the ventral and lateral surfaces of the duodenum and extends as far down as the umbilical region, well beyond the level of origin of the hepatic diverticulum. In the 6·0, 7·75 and 9·5 mm. embryos the ventral mesentery has become much thinner and its attachment is confined to the right and dorsal surfaces of the duodenum. It extends caudal to the biliary opening but does not reach the umbilicus.

Group II. 14–60 *mm. crown-rump length embryos*

In this group the formation of the horizontal part of the duodenum has been completed whereas, in embryos of group I, the caudal duodenum lies entirely to the right of the midline and a duodeno-jejunal flexure is absent. In the 14·0 and 18·0 mm. embryos the caudal duodenum is bent to form the horizontal portion which now extends as far as the midline before turning ventrally to continue as the proximal limb of the midgut loop. Even at this stage the duodeno-jejunal flexure is indistinct. From now onwards the horizontal part of the duodenum increases in length and reaches well over to the left of the midline so that, in the 26·0 and 37·0 mm. embryos, the flexure between the distal end of the duodenum and the proximal jejunum becomes increasingly clear. In these two embryos the horizontal part of the duodenum lies along the caudal surface of the funnel-shaped midgut mesentery (Text-fig. 1 *a*, *b*), and is only a short distance away from the vertebral bodies close to the root of this mesentery. As a result of these growth changes the midgut mesentery crosses ventral to the horizontal part of the duodenum with which it becomes fused from the 14·0 mm. embryo onwards. Furthermore, in the 37·0 mm. embryo, the caudal duodenum attains its characteristic position preparatory to rotation of the midgut loop as it returns to the abdominal cavity. The midgut is extra-abdominal in all embryos up to 37·0 mm. but is within the abdomen in the 45·0 mm. embryo in which the rotation of the midgut has been completed.

The epithelial outline of the duodenum, in transverse sections of the 14·0 mm. embryo, appears to be flattened from side to side and the bile duct now opens into the dorsal part of the left wall while the dorsal pancreatic duct enters its ventral part. The epithelial proliferation has now reached its maximum development and only small clefts remain in place of the duodenal lumen (Pl. 1, fig. 4). It was not possible to determine whether or not these clefts formed a continuous channel. The epithelial proliferation, which in the 9·5 mm. embryo was confined to those portions of the duodenum adjoining the biliary opening, now extends along its entire length. In the 18·0 mm. embryo the lumen of the duodenum is clearly re-established but traces of epithelial proliferation still persist in those parts of the duodenum adjacent to the duct orifices. The duodenal epithelium is of the stratified columnar type in the



Text-fig. 1. (*a*) The midgut and its mesentery in a 37·0 mm. embryo as viewed from the right (modified from Frazer, 1931). (*b*) Schematic drawing of section through root of midgut mesentery in a 37·0 mm. embryo (modified from Frazer, 1931).

B.	Bladder	*M.G.M.*	Midgut mesentery
H.D.	Horizontal part of duodenum	*M.G.Mc.*	Cut surface of midgut mesentery
H.G.	Hind gut	*S.*	Stomach
H.G.M.	Hind gut mesentery	*U.C.*	Umbilical coelom
M.G.	Midgut		

18·0 and 26·0 mm. embryos, the latter being the last to show remnants of the proliferation. In the 37·0 mm. embryo, the epithelium forms a single layer of columnar cells but a few patches of multi-layered columnar cells persist in places.

The proximal third of the dorsal mesentery of the duodenum, in the 14·0 and 18·0 mm. embryos, is attached to the dorsal surface of the duodenum. In the 26·0 mm. embryo an extension of the bursal cavity splits this part of the mesentery. In the middle third of the duodenum the dorsal mesoduodenum is greatly increased in thickness and contains the pancreas. The caudal third of the duodenum, which shares the common midgut mesentery, appears to grow round the root of this mesentery in such a way that the major part of the midgut mesentery, passing towards the extra-abdominal loops of gut, crosses ventral to this part of the duodenum (Text fig. 1*a*, *b*). This growth of the duodenum to the left and around the midgut mesentery progresses up to the 37·0 mm. stage. By this stage most of the root of the midgut mesentery lies on the ventral surface of the horizontal part of the duodenum and is also fused to it (Pl. 2, fig. 7).

In the 14·0 mm. embryo the ventral mesentery is attached to the dorsal surface of the proximal part of the duodenum and, in fact, both dorsal and ventral mesenteries converge and fuse with each other at their attachment (Pl. 1, fig. 5). The free margin of the ventral mesentery which, in the younger embryos of this group, extended caudal to the structures emerging from the liver is gradually brought towards its definitive position so that, in the 37·0 mm. embryo, the portal vein and the bile duct lie within the free margin of the ventral mesentery.

In the 37·0, 45·0 and 60·0 mm. embryos that part of the duodenum in relation to, and immediately caudal to, the opening of the bile duct bulges into the peritoneal cavity so that its lateral surface as well as portions of its dorsal and ventral surfaces are covered by peritoneum (Pl. 2, figs. 7, 9). Caudal to this region the ventral surface of the duodenum, which was covered by peritoneum in the 37·0 mm. embryo is now adherent to the proximal colon and its mesentery. The ventral surface of the horizontal duodenum is covered by the midgut mesentery as in the earlier embryos. No retention bands are seen in embryos of this group.

The 46·0 mm. embryo is quite remarkable in that the duodenum lies almost entirely on the right side of the abdomen and to the right of the midgut mesentery, as in the 9·5 mm. embryo. The duodenum and its mesentery, at the level of the bile duct, are laid against the posterior abdominal wall to which they are partly fused (Pl. 3, fig. 10). The bile duct enters the ventral portion of the duodenum but the rest of its course is normal. The duodeno-jejunal flexure is ill-defined and the coils of small gut occupy the dorsal half of the left side and the major portion of the right half of the abdomen (Pl. 3, fig. 11). The large gut lies ventrally in the left half of the abdominal cavity. The caudal duodenum has a long mesentery. It is thus evident that the terminal rotation of the midgut loop through 180° has failed and, had this embryo gone to full term, it is likely that there would have been only partial mid-gut rotation.

In the 14·0 mm. embryo the myoblasts of the circular muscle layer of the duodenum are distinct and stain deeply with eosin. In the 26·0 mm. embryo the circular muscle coat is well developed. The longitudinal muscle layer of the duodenum is first seen, in the 37·0 mm. embryo, as scattered strands in the duodenal wall immediately subjacent to its peritoneal covering (Pl. 2, fig. 8). The longitudinal muscle becomes increasingly clear from the 45·0 mm. embryo onwards.

Group III. 78·0–99·0 *mm. crown-rump length embryos*

The two embryos of this group possess an ascending part of the duodenum, but it is not clear how this part is formed. It was, however, noted that there was an appreciable increase and 'loosening up' of the mesenchyme around the duodenum from the 45·0 mm. stage onwards. It may be that, by this process, a part of the small gut which represents the proximal jejunum in the 37·0 mm. embryo is being absorbed within the expanding mesenchyme to form the ascending part of the duodenum in group III embryos.

These two embryos also illustrate the process whereby the duodenum is ultimately fixed to the posterior abdominal wall. As mentioned in the previous paragraph, the increase of the loose mesenchyme begins to obliterate the coelomic gutter which, in the 45·0 mm. embryo, intervened between the bulging walls of the vertical part of the duodenum and the posterior abdominal wall (Pl. 2, fig. 9). The progressive obliteration of the coelomic gutter proceeds from the medial side of the gutter towards its lateral side (Pl. 3, figs. 12, 13). A second peritoneal gutter, related to the distal end of the horizontal and ascending portions of the duodenum, likewise becomes gradually filled in by mesenchymal tissue. The process adopted is essentially similar to that occurring in the vertical segment of the duodenum in relation to the biliary opening.

DISCUSSION AND CONCLUSIONS

The above description of duodenal development confirms the findings of Frazer (1919) and Pernkopf (1925). Neither of these authors, however, laid any emphasis on the early fusion of the midgut mesentery to the ventral surface of the horizontal part of the duodenum, although it had been observed by Bardeen in 1914. Instead, most investigators have looked for some mechanism whereby the caudal part of the duodenum is retained inside the abdomen. Frazer & Robbins (1915) and Hunter (1927) sought to explain this intra-abdominal retention of the duodenum on the basis of retention bands. Frazer & Robbins (1915) and, subsequently, Frazer (1927, 1931) considered that these retention bands were merely condensed mesenchyme. Furthermore, Frazer (1927) was unable to establish the presence of the proximal retention band in the 22·0 mm. embryo which he examined. The early fusion of the midgut mesentery to the ventral surface of the horizontal part of the duodenum is not only capable of retaining the duodenum inside the abdomen, but may even promote the growth of the caudal duodenum in a transverse direction.

The bile duct. In the 4·0 mm. embryo the site origin of the hepatic diverticulum, which represents the future opening of the bile duct, is situated on the ventral aspect of the duodenum. In the 14·0 mm. embryo, however, the bile duct is attached to the medial wall of the duodenum and so is close to its definitive site in the adult. Keith (1948) suggests that this change in the position of the biliary opening may be due to rotation of the corresponding part of the duodenum and its mesentery through 90 degrees, thus bringing the bile duct to the right and the dorsal mesoduodenum towards the posterior abdominal wall. Differential growth of the duodenal wall is then invoked to move the biliary opening to its final position. It is clear from the present observations, supported by those of Pernkopf (1925), that the part of the duodenum bearing the biliary opening becomes retroperitoneal in a manner distinct from peritoneal fusions occurring elsewhere. In the normal embryo, rotation of this part of the duodenum, along with its mesentery, is unlikely to occur owing to the spiral growth of the caudal duodenum round the root of midgut mesentery. When, however, this process is arrested and the entire duodenum lies on the right side of the abdomen, as in the abnormal 46·0 mm. embryo, the duodenum is able to swing to the right and so becomes apposed to the posterior abdominal wall (Pl. 3, fig. 10). In this instance, the abnormal rotation carries the biliary opening to the ventral surface of the duodenum, a feature absent in all the normal embryos.

Hitherto, the epithelial proliferation in the duodenum has been unaccounted for, but it is significant that the proliferation is more marked in and around the biliary opening. This was also found by Johnson (1910). Indeed, in the younger embryos of this series, thickening of the epithelial wall follows closely the changes in position of the entrance of the bile duct. In the 14·0 mm. embryo the opening of the bile duct is actually occluded by the proliferated epithelial cells. It is, therefore, suggested that the epithelial proliferation contributes to the migration of the biliary opening. The epithelial thickening observed by Johns (1952) in the oesophagus is probably of a different nature since here it was effected without a corresponding increase in the number of epithelial layers.

Ventral mesentery. It is generally assumed that the changes in the ventral mesogastric attachment are brought about by a dextro-rotation of the duodenum. But the attachment of the dorsal and ventral mesenteries to a common site on the dorsal wall of the duodenum, in the 14·0 mm. embryo, cannot easily be reconciled with this theory of rotation. It is, therefore, more probable that changes in the ventral mesenteric attachment to the duodenum occur in a manner similar to that observed by Kanagasuntheram (1957) for the mesogastric attachment, i.e. by an extension of the peritoneal coelom into the compound mesoderm of the duodenal wall and ventral mesentery.

Rotation of the midgut. In the 6·0 mm. embryo the midgut has cranial and caudal limbs. By the 9·5 mm. stage the midgut loop has right and left limbs and so dextro-rotation through 90 degrees has already taken place. The displacement of the duodenum to the right is said to be due to the presence of the large vitelline vein, occupying the major part of the duodenal mesentery, and by growth of the pancreas (Frazer, 1919, 1931). Perhaps growth of the liver, which occupies the major part of the right half of the abdomen and the attachment of the ventral mesentery to this part of the liver, may also contribute towards the displacement of the duodenum to the right.

Prior to further rotation of the midgut loop through another 180 degrees, the caudal duodenum has grown round the root of the midgut mesentery so that this mesentery lies ventral to the caudal duodenum. It is not clear how this particular pattern of duodenal growth is determined; but whatever may be the cause, it is probable that the peculiar disposition of the caudal duodenum brings about the final rotation of midgut loop. This conclusion is supported by the findings in the 46·0 mm.

embryo in which the duodenum has not reached its normal position and the final rotation through 180 degrees has not occurred. Some of the anomalies of arrested intestinal rotation, in which the duodenum lies to the right of the midline (Grob, 1953), provide further support for this concept.

Various theories have been advanced to account for the return of the midgut loop (Frazer & Robbins, 1915; Pernkopf, 1925; Enbom, 1939). The fact that the longitudinal musculature is differentiated earlier in the small intestine than in the large gut and the evidence of Hamilton *et al.* (1952) that intestinal peristalsis is first noticeable at about the 40·0 mm. stage suggest that the contraction of the longitudinal musculature of the duodenum and proximal jejunum may well play a part in the return of the midgut loop. This would also account for the precedence of the proximal limb over the distal during the return of the umbilical portions of the gut.

SUMMARY

1. Some aspects of the development of the human duodenum in embryos of 3·8—99·0 mm. C.R. length are described.

2. The epithelial proliferation in the duodenum appears to be related to the migration of the biliary opening from a ventral position to its definitive site on the medial wall of the duodenum.

3. The changes in the attachment of the ventral mesentery to the duodenum appear to be brought about by an extension of the peritoneal coelom into the compound mesoderm of the duodenal wall and ventral mesentery and not by an actual dextro-rotation of the duodenum.

4. The duodenum becomes retroperitoneal as a result of increase in the surrounding mesenchyme.

5. There are no retention bands in relation to the distal part of the duodenum.

6. The final rotation of the midgut loop through 180 degrees is considered to be dependent upon the position of the terminal part of the duodenum in relation to the root of the midgut mesentery.

7. It is suggested that the withdrawal of the midgut loop into the abdomen is due to contraction of the longitudinal musculature of the duodenum and proximal jejunum.

It is a pleasure to thank Prof. J. D. Boyd not only for his interest during the progress of this work but also for putting at my disposal his collection of human embryos. I wish to express my gratitude to Prof. H. Butler for reading through the manuscript and for offering some valuable criticisms. I would also like to thank Mr J. F. Crane for the photomicrographs and Mrs R. Kanagasuntheram for Text-fig. 1*a* and *b*.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Transverse section through the 3·8 mm. embryo showing the dorsal pancreatic bud. Note broad apposition of septal mesoderm (future ventral mesentery) to duodenum. × 84.

Fig. 2. Transverse section through duodenum of the 6·0 mm. embryo to show origins of dorsal and ventral pancreases. Note small size of lumen of duodenum. The ventral mesentery is attached to right surface of duodenum and shows emergence of vitelline vein from liver. × 84.

Fig. 3. Transverse section through duodenum of 9·5 mm. embryo showing the openings of the dorsal pancreatic and bile ducts at same level. Note thinning of ventral mesentery. × 84.

Fig. 4. Transverse section through duodenum of the 14·0 mm. embryo, at the level of the biliary opening to show epithelial proliferation. Circular muscle coat is recognizable. × 210.

Fig. 5. Transverse section through duodenum of the 14·0 mm. embryo showing the portal vein emerging from the liver in the ventral mesentery. This mesentery is fused with the dorsal mesentery which is attached to the dorsal surface of duodenum. × 45.

Plate 2

Fig. 6. Transverse section through the abdominal region of the 14·0 mm. embryo to show fusion of midgut mesentery to caudal part of duodenum. × 18.

Fig. 7. Transverse section through the abdominal region of the 37·0 mm. embryo showing umbilical portion of midgut loop. Note that portions of the duodenal wall bulge into the peritoneal cavity. × 6.

Fig. 8. Transverse section through the wall of the caudal part of the duodenum to show the differentiation of longitudinal muscle layer. × 780.

Fig. 9. Transverse section through 45·0 mm. embryo to show the increase in mesenchymal tissue which tends to bring the duodenum to a retroperitoneal position. × 18.

PLATE 3

Fig. 10. Transverse section through duodenum of the abnormal 46·0 mm. embryo to show fusion of duodenum to the posterior abdominal wall. Note the pancreas lying directly ventral to the duodenum. × 18.

Fig. 11. Transverse section through abdomen of 46·0 mm. embryo to show incomplete rotation of midgut loop. Note loops of large gut occupying the left and ventral part of the abdomen. × 6.

Fig. 12. Transverse section through duodenum of 60·0 mm. embryo to show increase in mesenchyme which brings about the retroperitoneal position of duodenum. Note gutter of peritoneal cavity between caudal part of duodenum and descending mesocolon. × 6.

Fig. 13. Transverse section through 99·0 mm. embryo to show how the gutter of peritoneal cavity on left side is filled in by mesenchyme. The duodenum on the right side is already retroperitoneal. Note that the proximal colon is fused to ventral surface of duodenum. × 6.

Key to lettering of plates

A.	Aorta	*L.*	Liver
B.D.	Bile duct	*L.M.*	Longitudinal muscle layer
C.	Colon	*L.S.*	Lesser sac
Ce.	Caecum	*M.G.M.*	Midgut mesentery
C.M.	Circular muscle layer	*P.*	Pancreas
D.	Duodenum	*P.G.*	Peritoneal gutter
D.C.	Descending colon	*P.V.*	Portal vein
D.L.	Duodenal lumen	*S.*	Stomach
D.M.	Dorsal mesoduodenum	*S.R.*	Suprarenal gland
D.P.	Dorsal pancreas	*T.C.*	Transverse colon
D.P.D.	Dorsal pancreatic duct	*U.C.*	Umbilical coelom
E.P.	Proliferated epithelium	*V.P.*	Ventral pancreas
K.	Kidney	*V.V.*	Vitelline vein

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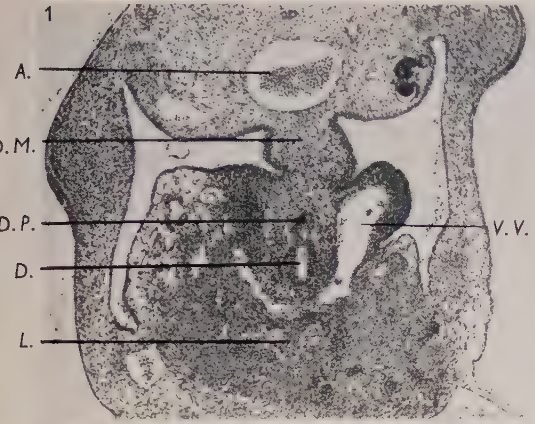

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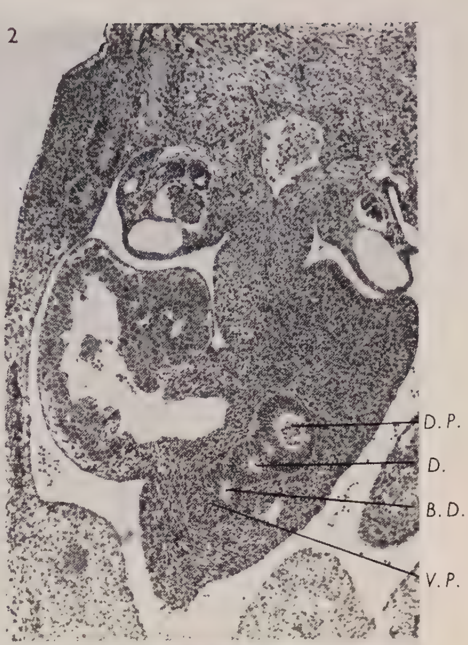

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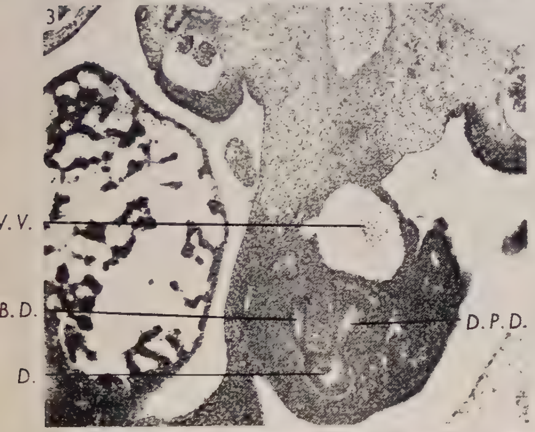

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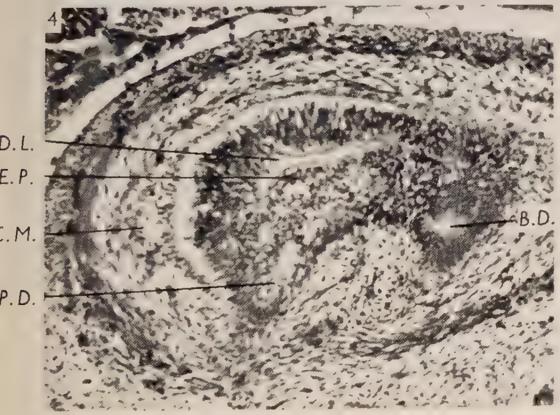

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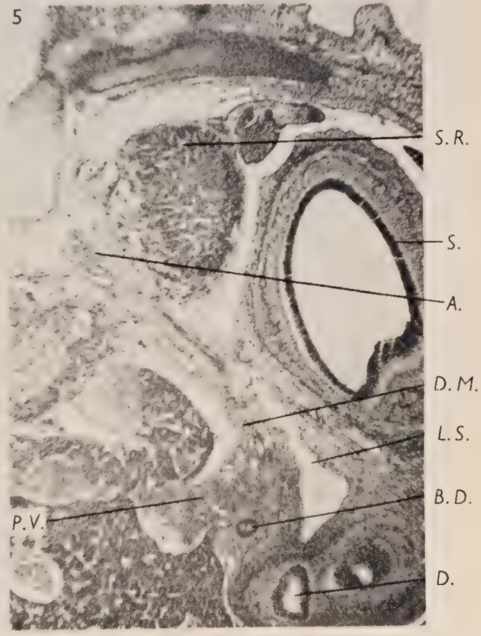

KANAGASUNTHERAM—DEVELOPMENT OF THE HUMAN DUODENUM

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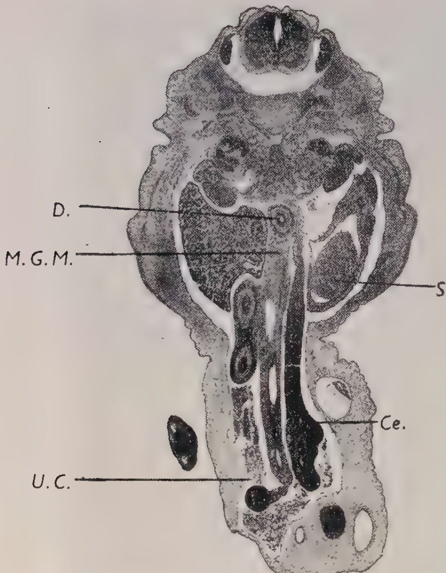

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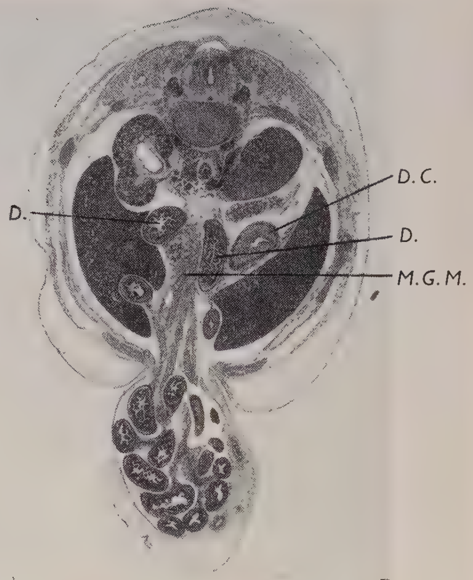

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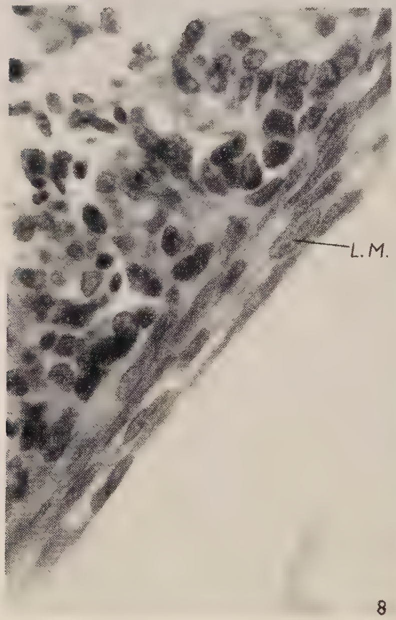

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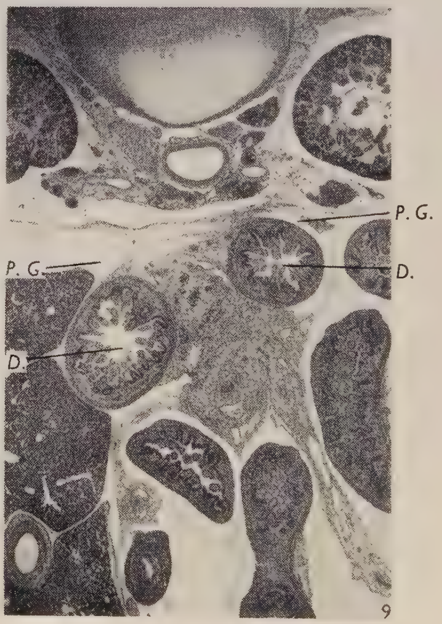

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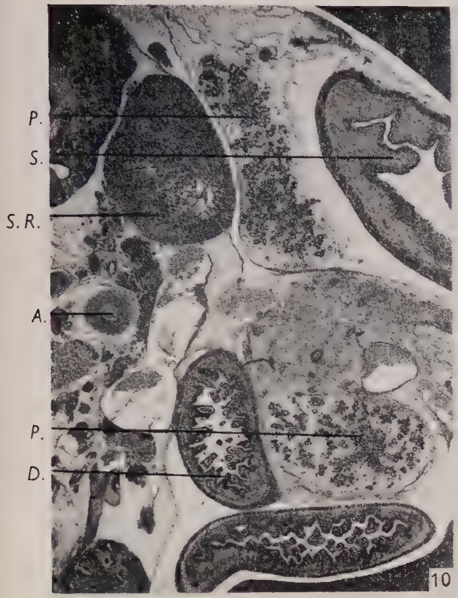
KANAGASUNTHERAM—Development of the human duodenum



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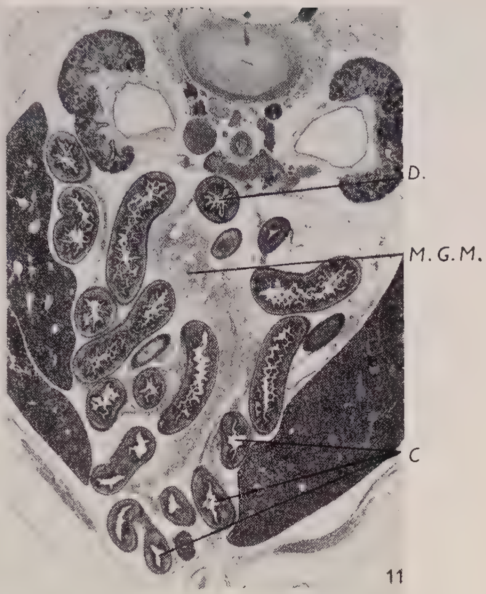

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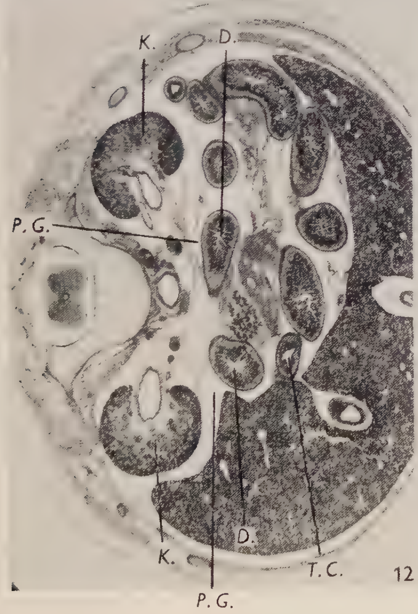

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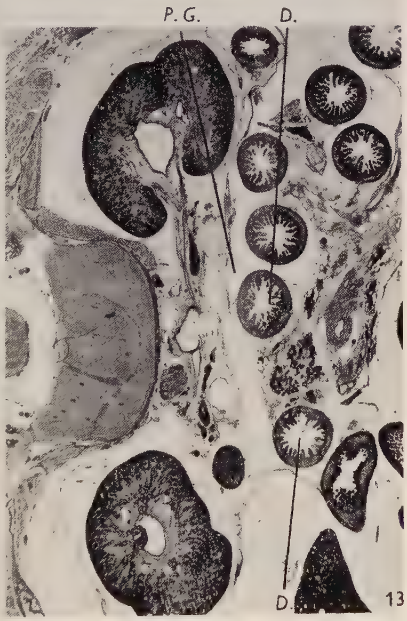

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KANAGASUNTHERAM—Development of the human duodenum

THE EFFECT OF POST-MORTEM AUTOLYSIS ON SYNAPTIC TERMINALS IN CEREBRAL CORTEX OF DOG

By J. R. SMYTHIES* and O. R. INMAN†

The nature of the *boutons termineaux* in the cerebral cortex has been subject to conflicting reports. Smythies, Gibson & Purkis (1957*b*) were unable to observe typical *boutons termineaux* (adjacent to neuron cell membranes as seen in the spinal cord and globus pallidus) in the human cerebral cortex using a silver stain. Armstrong & Young (1957), however, using a stain specific for mitochondria, could demonstrate many *boutons* surrounding the cortical neurones of cat. Now it is a well-known fact that terminals are more easily stained by silver methods when they are in a state of degeneration (see, for example, Smythies, Gibson, Purkis & Lowes, 1957*a*). It therefore occurred to us that the degeneration produced by post-mortem autolysis might also enable us to stain these structures more easily with a silver method.

MATERIAL AND METHODS

The brains of six dogs were used in this study. In the first, the whole brain of the living anaesthetized dog was perfused with saline followed with formol-saline and removed. In the second, a hemispherectomy was very kindly performed by Dr Stanton Goldstein on the living anaesthetized dog and the remainder of the living brain was immediately perfused. The portion removed was allowed to stand at room temperature for 1 hr. before fixation by slicing and immersing in formalin. In the rest, the animal was killed and the brain immediately removed and quartered. One quarter was fixed after 1 hr., another after 24 hr., another after 48 hr. and the last after 72 hr. post-mortem autolysis at room temperature. Or else the brain was halved and the two halves fixed at different times. The slices were fixed in formalin for 6 months and then frozen sections were stained by the Rio-Hortega double impregnation technique as described by Smythies *et al.* (1957*b*). Six sections were cut and stained from each of the following locations—frontal pole, premotor cortex, Betz cell region, sensory cortex and occipital pole. The sections were examined by regular scanning under the oil-immersion lens.

(1) 0 *hr. post-mortem autolysis*. The sections from all loci show well-fixed neurons and glia together with a dense neuropil and no trace whatever of any *boutons termineaux* (Pl. 1, fig. 1).

(2) 1 *hr. post-mortem autolysis*. The neuropil here was dense, but the fibres were somewhat more irregular with occasional thickenings and irregularities along their course. No *boutons* were seen except around a few of the Betz cells (Pl. 1, fig. 2), where well-defined *boutons* were stained. These were somewhat irregular in shape with some fibrillary internal structure, clearly connected to preterminal fibres, and they tended to lie in chains in close apposition to the surface of the Betz cell.

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These terminals were well stained in only one brain and were only poorly shown in two others, including the case operated upon. However, no trace of such structures were ever seen in either of the two perfused brains.

(3) 24 *hr. post-mortem autolysis.* The change here in terminal structures was quite striking. The neurones presented clear-cut evidence of autolysis and the neuropil had almost completely fragmented (or was no longer stainable). A few fibres were still seen (Pl. 1, fig. 3) coursing amid much debris—small globular and irregular masses staining to a variable degree but mainly rather faintly. However, around many cells—particularly the large pyramidal cells of layers 3 and 5 of the motor cortex—the condition illustrated around the cell in the centre of Pl. 1, fig. 3 was seen. Many globular or more or less regular masses staining darkly were closely packed around the cell. These structures were also seen scattered rather sparsely around some of the larger cells in the sensory cortex and premotor region but there were none to be seen at the frontal or occipital poles.

(4) 48 *hr. post-mortem autolysis.* The changes seen at 24 hr had progressed even further. The neuropil was almost completely fragmented with many granules scattered between the cells but particularly surrounding the large pyramidal and Betz cells of the motor region. Here the surface of the neurone was covered with small, irregular and densely staining bouton-like objects (Pl. 1, fig. 4). They also surrounded many more cells, both large and small, than at 24 hr. and were also plentiful in the sensory region. A few were also seen around larger cells at the frontal and occipital poles.

(5) 72 *hr. post-mortem autolysis.* The brain was too softened at this stage for any adequate sections to be cut.

DISCUSSION

These findings suggest a progressive change in the appearance (as revealed by this silver stain) of the terminal apparatus of axons surrounding neurons in the cerebral cortex brought about by post-mortem autolysis. These changes are most marked around larger cells and regionally in order—motor cortex, premotor and sensory, occipital and frontal poles. This may be interpreted in two ways: (i) *boutons* present normally cannot be stained by the silver method used unless they are undergoing the chemical changes of post-mortem autolysis (or other degenerative conditions), or (ii) the structures here stained may not be *boutons* but may be pathological products of the fragmentation of the neuropil around cells affected by autolysis. In this case we may suppose that the neuropil degenerates throughout the cortex, as these sections show it does, but that the degenerative products stain better around cells. This latter effect might be due to some chemical specificity of the fibres in their terminal or paraterminal sections or it might be due to the diffusion of some chemical products of autolysis from the neighbouring cell body. At present there seems to be no evidence to help determine this point. The former would appear, however, to be the more probable alternative.

SUMMARY

The effect of post-mortem autolysis (0, 1, 24 and 48 hr.) on the appearance of *boutons termineaux* in the cerebral cortex of dog has been investigated using a silver-staining method. It was found that the brain perfused during life shows a

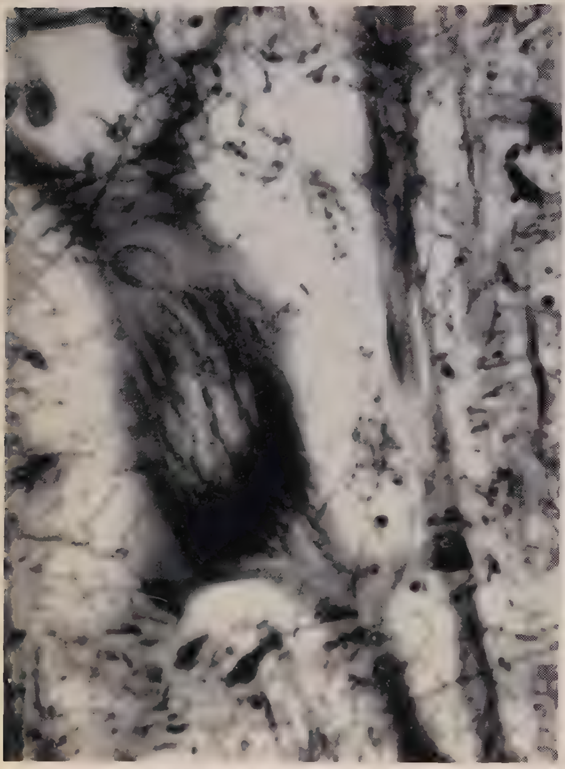
Fig. 1

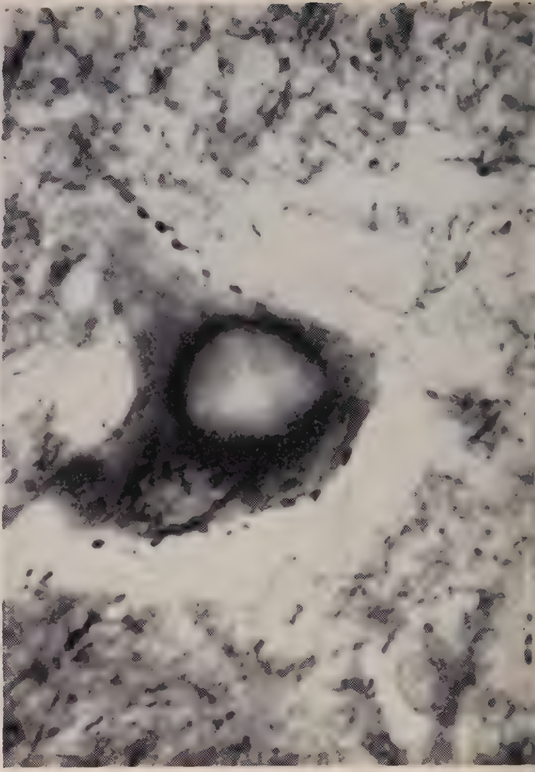
Fig. 2

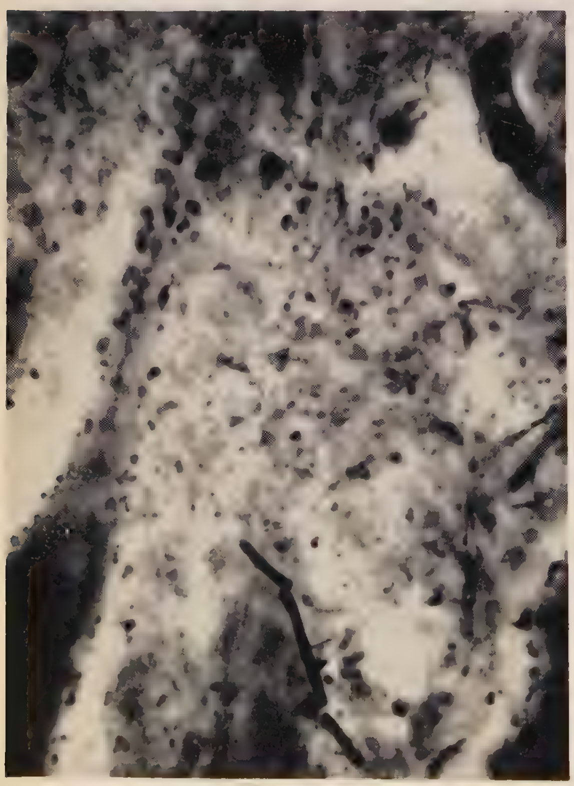
Fig. 3

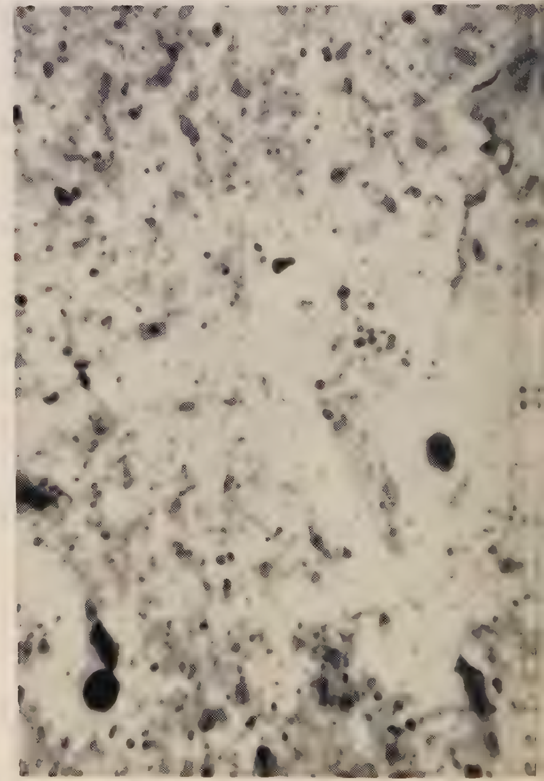
Fig. 4

SMYTHIES and INMAN—Effect of post-mortem autolysis on synaptic terminals of dog

dense neuropil but no *boutons*. In the brain that has undergone 48 hours post-mortem autolysis the neuropil has fragmented and many small darkly-staining bodies resembling *boutons termineaux* are seen closely surrounding the neuron. The changes are most prominent in the case of the larger cells of the motor cortex and were hardly seen at the occipital and frontal poles. Intermediate degrees of post-mortem autolysis produced intermediate pictures between these two. The significance of these findings is discussed.

We are most grateful to Dr Williamina Himwich for the provision of material and her interest in this research.

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EXPLANATION OF PLATE 1

Fig. 1. A Betz cell in the cortex of a brain perfused during life. (× 3,000.)

Fig. 2. A Betz cell in a brain that has undergone 1 hr. post-mortem autolysis. (× 3,000.)

Fig. 3. The apical dendrites of two Betz cells in a brain after 24 hr. post-mortem autolysis. Portions of other cells and a fragmenting neuropil are also seen. Some putrefactive bacteria are seen in the lower part of the figure. (× 3,000.)

Fig. 4. A pyramidal cell together with a fragmenting neuropil in layer 5 of the motor cortex of a brain after 48 hr. post-mortem autolysis. (× 3,000.)

THE POST-NATAL DISTRIBUTION OF THE ABDOMINAL CHROMAFFIN TISSUE IN THE GUINEA-PIG, MOUSE AND WHITE RAT

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Groups of extra-adrenal chromaffin cells were observed by Stilling (1890) in association with the abdominal sympathetic plexuses of the cat, rabbit and dog and these cells were shown subsequently to resemble those of the adrenal medulla, not only in their affinity for chrome salts, but also in that pressor amines may be extracted from both elements (Biedl & Wiesel, 1902).

Much of the earlier work on the extra-adrenal chromaffin tissue was confined to the study of this material in man, and yet although human post-mortem material is satisfactory for determining the general topographical distribution of this tissue, it is often unsuitable for detailed cytological observations as a result of the ante-mortem, agonal and post-mortem changes which have affected the cells. Phaeochromocytes are highly reactive to a variety of stimuli. These may result from either starvation (Jackson, 1919) or the administration of hormones or drugs (Cramer, 1928; Hillarp, 1946; Coupland, 1959). Intense stimulation usually results in an increase in nuclear basophilia with some shrinkage and in cytoplasmic vacuolation. These changes may therefore be readily interpreted as signs of cellular degeneration in spite of the fact that they are reversible.

The earlier works on the distribution of chromaffin tissue in man have been summarized previously (Coupland, 1952, 1954). It is now generally accepted that much of the extra-adrenal chromaffin tissue disappears from its normal site during childhood, at between 3 and 7 years of age, and at a time when the adrenal medulla is approaching full development. The reason for this change is not as yet clear and the study of this problem in man is limited by the difficulty of obtaining really fresh material which is suitable for cytological observations. This problem has therefore been investigated in a number of lower mammals and for this work members of the cohort Glires have been used since they are readily obtainable and, in addition, many contain large amounts of extra-adrenal chromaffin tissue. Furthermore, at least one member of this cohort, the mouse, has been reported to possess extra-adrenal chromaffin bodies which disappear during the pre-pubertal phase (Goormaghtigh, 1935); a change which has some resemblance to that which occurs in man. According to Goormaghtigh (1935) the disappearance of these cells from the pre-aortic region is the result of a peripheral migration.

Detailed descriptions of the extra-adrenal abdominal chromaffin tissue of the rabbit already exist (Kohn, 1903), Coupland (1956). Only brief mention has, however, been made to this material in the guinea-pig, mouse and rat. Vincent (1910) was unable to demonstrate extra-adrenal chromaffin tissue in the guinea-pig, but its presence in this mammal was observed by Wislocki (1922). The only reference to this material in the rat would appear to be a letter from Kohn to Vincent (1910) in which Kohn stated that he had occasionally observed a discrete extra-adrenal chromaffin body in this animal. Vincent was, however, unable to find any evidence of para-aortic chromaffin bodies in the white rat.

MATERIALS AND METHODS

Guinea-pigs, strong A strain albino mice and Wistar strain rats were used. Guinea-pigs were aged 1, 2 and 5 days; 4 (two), 10, 14, 24 (two), 36 (two) and 80 weeks; two 6·5 cm. foetuses were also examined. Mice were aged 1 (three), 10, 14, 19, 21 (two), and 28 days (two); 8, 12 (two), 16 and 36 weeks (two); three 18 mm. foetuses were also examined. Rats aged 1 (two), 7 (two), 14 and 28 days were examined. Many of the specimens were litter-mates. Animals were killed by a blow on the head. The majority were fixed by perfusion with formol-dichromate (neutral formaldehyde 5%, potassium dichromate 3%) followed by immersion of blocks of the posterior abdominal wall and associated viscera in the fixative for 24 hr. The tissues were then washed, dehydrated and embedded in paraffin. Serial sections were made at 6–10μ. Blocks obtained from the larger animals were cut into conveniently sized pieces after fixation but before embedding. Some specimens were fixed in Carnoy's fluid (6:3:1), Helly's fluid, formol-Zenker or Bouin's fluid. In most instances alternate slides were stained by Ehrlich's haematoxylin and Giemsa's stain. A few slides were stained by one of the following stains: iron haematoxylin, Mallory's trichrome, Feulgen's leucofuchsin or methyl green-pyronin. Graphic reconstructions of a number of specimens were made, using a camera lucida, in order to reveal the position of the chromaffin cells in relation to the abdominal aorta.

RESULTS

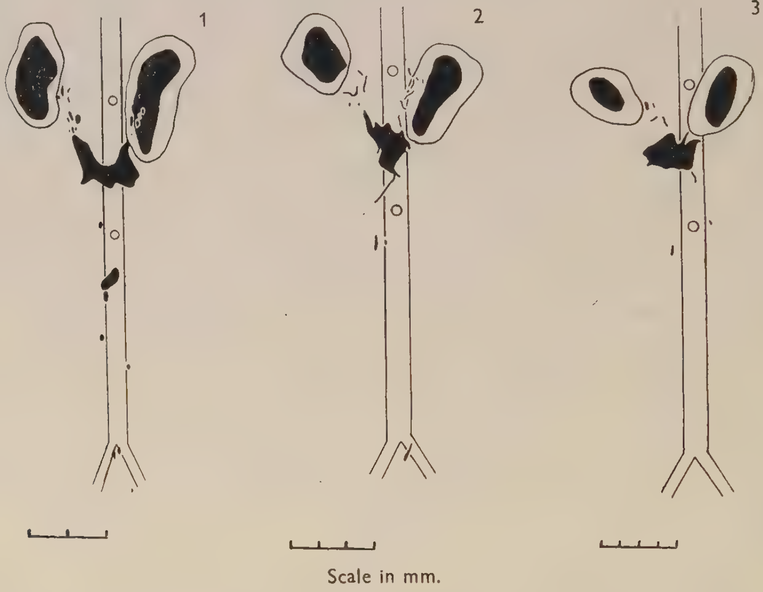
As no sex difference has been observed in the distribution of chromaffin tissue during the present work, the sex of the animals will not normally be specified.

GUINEA-PIG

6·5 cm. foetus

A well-marked chromaffin reaction is observed in the cells of the adrenal medulla and in cells associated with the abdominal sympathetic nerve plexuses. At this stage the adrenal medulla is relatively large and accounts for one-third of the diameter of the gland; it is composed mainly of chromaffin cells, but also contains sympathetic neurones which may be observed singly or in clumps. These neurones are occasionally associated with collections of small, darkly staining cells, which resemble small lymphocytes.

Extra-adrenal chromaffin cells occur singly or in groups in the pre-vertebral sympathetic ganglia. The largest collection of these cells lies between the origin of the superior and inferior mesenteric arteries (Text-fig. 1, Pl. 1, fig. 1) and measures from 1 to 2 mm. in cranio-caudal and transverse diameters. It is intimately associated with the fibres of the pre-aortic and renal sympathetic plexuses. The rostral part of this structure passes posterior to the left renal vein (Pl. 1, fig. 2). Smaller chromaffin bodies occur in the coeliac, superior and inferior mesenteric ganglia. These are often elongated structures and measure up to 0·5 mm. in long axis. In the lumbar para-vertebral sympathetic ganglia chromaffin cells are occasionally observed either singly or in small groups of up to four cells. Some ganglia are devoid of these elements whilst others contain more than one cell or group of cells. The main extra-adrenal chromaffin body contains small groups of sympathetic neurones but, as in the adrenal medulla, chromaffin cells predominate. The sympathetic neurones contain nuclei which measure 8–10μ in diameter; nucleoli are prominent.



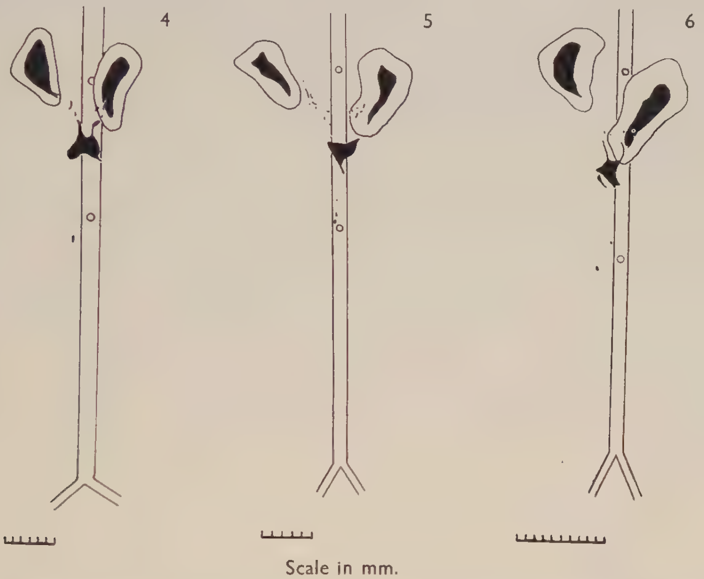
Text-figs. 1–3. Reconstructions of the chromaffin tissue of a 6·5 cm. guinea-pig foetus and post-natal specimens aged 1 day and 4 weeks, respectively. The aorta, with the origins of the superior and inferior mesenteric arteries, and the adrenal glands are also shown.

Irrespective of situation, each chromaffin cell has a round or ovoid nucleus which measures 6–8μ in maximum diameter and contains three or four scattered granules of chromatin; these usually lie immediately beneath the nuclear membrane. Supporting connective tissue stroma is only observed after the use of silver stains for reticular tissue. Mitotic figures have been observed in both the adrenal medulla and extra-adrenal chromaffin tissue, and in the two specimens examined they are seen with an average frequency of 1 in 6 sections.

1–5 *days old*

The distribution of chromaffin tissue is indicated in Text-fig. 2. As in the previous specimen the main chromaffin body lies in the pre-aortic region (Pl. 1, fig. 3) between the origins of the superior and inferior mesenteric arteries and in the day-old specimen measures 2·4 mm. in a cranio-caudal direction and 1·6 mm. in transverse diameter. Its rostral extremity is crossed anteriorly by the right renal vein. A close association between this body and the pre-aortic and renal sympathetic plexuses is again apparent and has indeed been observed in all specimens irrespective of age. Smaller and more elongated collections of chromaffin cells, which may measure up to 0·5 mm. in long axis, are present in the coeliac and superior mesenteric ganglia (Pl. 1, fig. 4, Text-fig. 2).

The chromaffin cells give a stronger positive chromaffin reaction than those of the foetal specimens and many contain small cytoplasmic vacuoles (Pl. 1, figs. 5, 6). Mitotic figures have been observed in the adrenal medulla but have not been seen in the main extra-adrenal chromaffin body (every fourth serial section was examined). The connective tissue stroma of the latter is more abundant than in the previous specimen and routine stains now demonstrate the presence of collagenous fibres. All extra-adrenal chromaffin bodies are surrounded by a thin connective tissue capsule; this is most prominent in the main body.



Text-figs. 4–6. Reconstructions of the chromaffin tissue of guinea-pigs aged 10, 24 and 36 weeks, respectively. The aorta, with the origins of the superior and inferior mesenteric arteries, and the adrenal glands are also shown.

Sympathetic neurones are often associated with chromaffin cells, both inside and outside the adrenal gland; they are larger than those of the previous specimen and contain a nucleus which measures 10–15μ in diameter.

4–80 *weeks*

In all these animals the pattern of distribution of chromaffin tissue is similar. Reconstructions of specimens aged 4, 10, 24 and 36 weeks are reproduced in Text-figs. 3–6.

Small encapsulated collections of phaeochromocytes which measure up to 0·5 mm.

in long axis, exist in the large pre-vertebral sympathetic ganglia and are most numerous in the coeliac and superior mesenteric collections of nerve cells. In each specimen the largest para-aortic chromaffin body conforms in position and relations to that of the earlier specimens but shows an increase in length and width (Text-fig. 13). These changes are associated with a reduction in the thickness of the body, as measured dorso-ventrally (Pl. 1, figs. 1, 3; Pl. 2, figs. 7, 9). Hence, with age the main body becomes wider, longer and flatter.

The extra-adrenal chromaffin cells are supported by a stroma which is rich in collagenous fibres. Collagen is most abundant in the main body and appears to increase in amount with age. In some of the small extra-adrenal chromaffin bodies, associated with the coeliac and superior mesenteric ganglia, blood-capillaries and thin walled veins form a conspicuous feature (Pl. 2, fig. 8).

No evidence of cellular infiltration of chromaffin tissue has been obtained in any of these animals and a well-defined healthy looking body still exists in the 80-week-old specimen (Pl. 2, fig. 9).

Phaeochromocytes have the same general structural features as in the earlier specimens and a positive chromaffin reaction is observed in both intra- and extra-adrenal cells, though in the adrenal medulla the reaction is usually stronger and of a darker brown variety than in the para-aortic bodies. Cytoplasmic vacuoles may be observed in any chromaffin cells but are usually larger in the extra-adrenal elements (Pl. 2, figs. 10, 11). In the adrenal medulla the cells are cuboidal or columnar in shape, are arranged around venous sinuses and are supported by a reticular stroma. In the para-aortic bodies, the chromaffin cells form cords in association with collagenous connective tissue and capillary blood vessels. Towards the lateral extremities of the main para-aortic bodies the chromaffin cells are seen to be aligned with their long axes running more or less transversely and parallel to the fibres of the renal sympathetic plexus; elsewhere they are arranged irregularly. Examination of each fourth section through the entire adrenal medulla and main para-aortic chromaffin body in specimens aged 10, 24 and 36 weeks has failed to reveal the presence of mitotic figures. There is no definite evidence of degeneration of these elements.

As in earlier specimens, chromaffin cells are occasionally observed singly or in small groups in the lumbar para-vertebral sympathetic ganglia: they are inconstant in position and some ganglia are free from these elements.

Small collections of ectopic adrenal cortex exist in specimens aged 10, 14 and 28 weeks. They lie near the upper poles of the kidneys and in the immediate vicinity of the adrenal gland.

MOUSE

18 *mm. foetus*

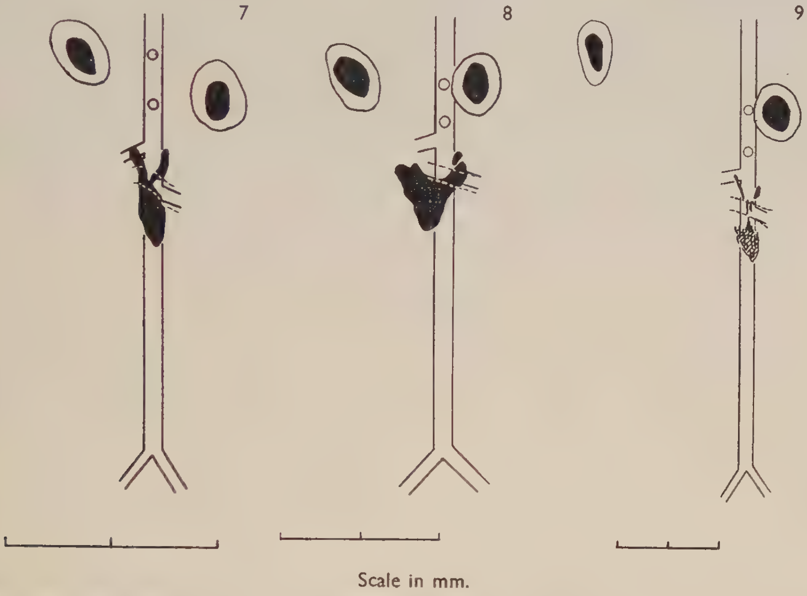
Sections through the adrenal glands show a well-defined cortex and medulla. The medulla occasionally reaches the surface at the lower medial aspect of the gland but is not continuous with extra-adrenal chromaffin tissue. The medullary cells give a very faint positive chromaffin reaction.

A compact mass of chromaffin tissue lies anterior to the aorta immediately caudal to the left renal vein, its upper extremities embracing the latter structure. The body lies inferior to the origin of the right renal artery, but overlaps that of the left renal artery and extends some 300μ below this level.

No extra-adrenal phaeochromocytes have been observed elsewhere at this stage, but this fact does not preclude the presence of small groups of these cells, since the chromaffin reaction is so faint that isolated cells or small aggregations could easily be overlooked.

1–19 *days old*

The distribution of chromaffin tissue is indicated in the reconstructions Text-figs. 7, 8.



Text-figs. 7–9. Reconstructions of the chromaffin tissue of mice aged 1, 10 and 21 days, respectively. The aorta (with origins of the coeliac, superior mesenteric and renal arteries), the left renal vein and the adrenal glands are also shown.

A positive chromaffin reaction, which increases in intensity from faint in the day-old specimen to moderately intense in the 19-day-old mouse, is given by the cells of the adrenal medulla and a large compact chromaffin body which lies anterior to the aorta (Pl. 2, fig. 12; Pl. 3, figs. 13–15). In the adrenal medulla the reaction is patchy and some groups of cells give a stronger reaction than others. In the extra-adrenal body the reaction is uniform throughout. Other smaller collections of up to four chromaffin cells occur inconstantly in the pre- and para-vertebral sympathetic ganglia. The greater part of the main extra-adrenal chromaffin body lies caudal to the left renal vein, while its upper extremity embraces this structure (Pl. 3, fig. 13).

The adrenal phaeochromocytes have rounded or ovoid nuclei 6–8μ in diameter which contain scattered granules of chromatin. The extra-adrenal cells are smaller and contain ovoid nuclei, which measure 5–7μ in diameter. In the 1- to 10-day-old animals all chromaffin cells are supported by a reticular connective tissue stroma but in the 14- and 19-day-old specimens collagenous fibres are present. Mitotic figures are present in both intra- and extra-adrenal chromaffin cells in all specimens and at 14 days have a frequency of 1 in 10 sections.

21 *days*

The chromaffin cells of the main para-aortic body extend over a greater distance in a cranio-caudal direction than in any previous specimen (Text-fig. 9). Some cells appear to have become detached from the rostral extremities of the body and others lying below the level of the left renal vein no longer form a compact mass (Pl. 3, fig. 16). Occasional pyknotic nuclei are observed in the chromaffin cells of the main body. Small para-aortic chromaffin bodies of less than 100 μ in diameter lie in the renal plexus adjacent to the hilum of the kidney on the right side, and in association with sympathetic neurones in the caudal part of the pre-aortic sympathetic plexus.

Vagal paraganglia have been observed in association with the nerve fibres of the superior mesenteric and coeliac plexuses. These are encapsulated structures composed of small cells whose nuclei measure 5–6 μ in diameter and contain prominent chromatin granules: the cytoplasm of these elements gives a negative chromaffin reaction.

4–36 *weeks*

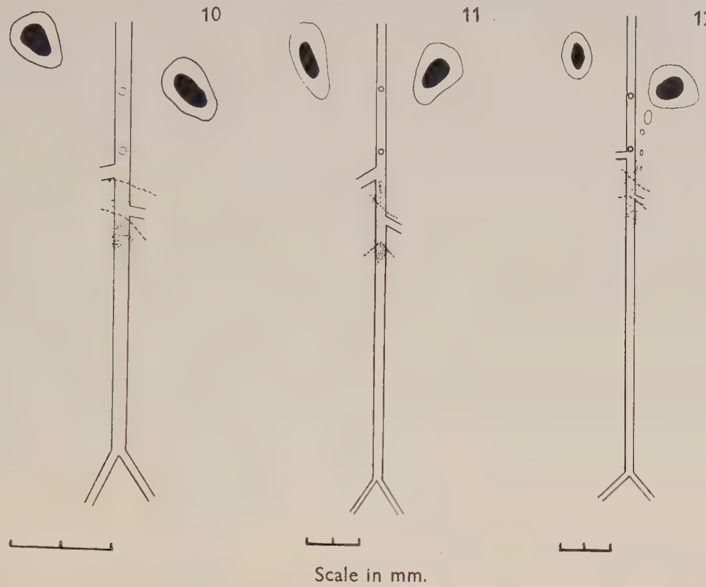
The disruption of the formerly compact extra-adrenal chromaffin body first observed in specimens aged 21 days becomes more marked as the age of the animal increases and in all these specimens the pre-aortic region rostral, dorsal and caudal to the left renal vein contains irregular groups or cords of chromaffin cells (Pl. 3, figs. 17, 18). These elements have not been observed extending along the distal ramifications of the pre-vertebral sympathetic plexuses and there is therefore no reason to suppose that a distal migration of chromaffin elements occurs. Small groups of up to three phaeochromocytes have been observed in the ganglia of the hypogastric plexus and of the lumbar sympathetic chain; they are, however, inconstantly present.

The chromaffin reaction is moderately intense in all situations but in the adrenal gland the intensity of the reaction varies from one group of cells to another; the reaction in the extra-adrenal elements is more uniform. The medullary phaeochromocytes contain rounded nuclei, 6–8 μ in diameter and have an abundant cytoplasm. The extra-adrenal cells contain round or ovoid nuclei 5–7 μ in diameter, cytoplasm being scanty. Nuclei in both situations contain scattered chromatin granules and nucleoli are often visible. An occasional pyknotic nucleus is observed in chromaffin cells of the pre-aortic region and although an accurate estimate of the number of cells present before and after the disintegration of the main extra-adrenal chromaffin body is virtually impossible because of the scatter of these elements, it would appear that in the 36-week-old specimen the number is less than in younger animals.

In a 12-week-old pregnant female specimen (Text-fig. 12) four masses of ectopic adrenal cortex lie caudal to the left adrenal gland. In one of these degenerative changes identical with those which affect the X-zone of the adrenal cortex may be observed.

RAT

In one 7-day-old specimen a small collection of chromaffin cells, 25μ in diameter, was observed in the pre-aortic sympathetic plexus immediately caudal to the superior mesenteric artery; in the others no extra-adrenal chromaffin cells were observed.



Text-figs. 10–12. Reconstructions of the chromaffin tissue of mice aged 4, 8 and 12 weeks, respectively. The aorta (with origins of the coeliac, superior mesenteric and renal arteries), the left renal vein, the adrenal glands, and, in Text-fig. 12, ectopic adrenal cortex are also shown.

DISCUSSION

During the present work six Wistar strain albino rats, aged 1–28 days, have been examined. In five of these animals no evidence of extra-adrenal chromaffin cells has been obtained, while in one a small discrete collection of these cells was observed adjacent to the superior mesenteric artery. It would appear, therefore, that in the abdomen of the rat the chromaffin tissue is virtually confined to the adrenal gland.

In both the new-born mouse and guinea-pig a large well-defined chromaffin body exists in the pre-aortic region. In the guinea-pig this persists throughout life, while in the mouse the structure begins to disintegrate in animals aged 3–4 weeks and only small scattered groups of chromaffin cells remain in older animals. No evidence of a true peripheral migration of these elements, as suggested by Goormaghtigh (1935), has, however, been obtained. In both the mouse and guinea-pig the main para-aortic chromaffin body is closely related to the left renal vein and its rostral extremity often embraces this structure. In the guinea-pig the body is triangular or rectangular in shape and is closely associated with both the obliquely running fibres of the renal plexus and with the longitudinal fibres of the pre-aortic sympathetic plexus. Its disposition and associations are therefore different from those of the main para-aortic bodies of man (Coupland, 1954) and rabbit (Coupland, 1956), which are associated only with the longitudinally running fibres of the pre-aortic plexus.

The enlargement of the main para-aortic body of the guinea-pig, between birth and 4 weeks old, is associated with an increase in supporting stroma, which changes from a reticular type, present in the foetus, to one in which collagenous fibres are evident. This change is similar to the one that takes place in the extra-adrenal chromaffin bodies of both man and rabbit and there is no reason to suppose that it is *per se* a sign of degeneration. The increase in stroma is often more apparent in perivascular sites than elsewhere, but endarteritis has never been observed. Indeed, capillary type blood vessels are often more apparent in older specimens, particularly in the rabbit and guinea-pig. The increase in perivascular fibrous tissue observed in man, rabbit and guinea-pig may, therefore, be an incidental phenomenon unrelated to the well being of the chromaffin elements, since identical changes may be observed in other vessels in the pre-aortic region.

Cellular infiltration of the para-aortic bodies has not been observed in the present work. This result is in keeping with the findings in man and rabbit (Coupland, 1954, 1956). Nuclear pyknosis has very occasionally been observed in the phaeochromocytes of both guinea-pig and mouse, and has been more commonly observed in the extra-adrenal cells than in those which form the adrenal medulla. The significance of nuclear pyknosis in chromaffin tissue is, however, difficult to assess, since, as noted above, cytoplasmic vacuolation and nuclear pyknosis can very readily be produced in chromaffin cells by a variety of stimuli. It does, however, appear likely that in the mouse, some of the pre-aortic chromaffin cells do undergo degeneration after the disintegration of the main chromaffin body.

During the present investigation and in previous works (Coupland, 1954, 1956) the detailed distribution of chromaffin tissue in the abdomen of man, rabbit, mouse and guinea-pig has been observed and the changes which affect this tissue during the growing phase, from birth to sexual maturity or later, have been noted. In all these animals, at birth and for a short time thereafter, a large number of phaeochromocytes exists in the pre-aortic region and forms a main extra-adrenal chromaffin body. Other small bodies may be associated with any part of the pre-vertebral sympathetic nervous system and chromaffin cells are not uncommonly observed in the ganglia of the lumbar sympathetic chain. During the pre-pubertal phase the main extra-adrenal chromaffin bodies show changes in disposition. In man the bodies (organs of Zuckerkandl) first elongate and then disintegrate during childhood, the constituent cells being scattered between the fibres of the pre-aortic sympathetic nerves. In the mouse the body first elongates and then disintegrates in the 3- to 4-week-old animal. In the rabbit the body merely elongates. In the guinea-pig the body increases in overall dimensions in both longitudinal and transverse directions. It should be noted that these changes affect only the largest para-aortic body, the smaller more compact masses persisting in both pre- and para-vertebral ganglia. This selectivity suggests that local factors may be involved in the changes which take place.

It has been generally accepted for many years that the chromaffin system reaches maturity at an early stage after birth (Elliott & Tuckett, 1906) and the time of occurrence of mitotic figures in both the adrenal medulla and para-aortic bodies during this investigation would support this contention. Extra-adrenal chromaffin bodies are encapsulated structures made up of phaeochromocytes, sympathetic nerve fibres, and a variable amount of connective tissue stroma. The main bodies lie with their long axes directed cranio-caudally and are in intimate association with the longitudinally running fibres of the pre-aortic sympathetic plexus. In the guinea-pig the main body is also closely associated with the more transversely disposed fibres of the renal plexus. The pre-aortic sympathetic nerve fibres will of necessity undergo a considerable increase in length as the animal grows: the change being roughly equal to that which affects the abdominal aorta. The growth of these nerve fibres must, therefore, be accompanied by a change in the structural form or arrangement of the contiguous chromaffin bodies. It is apparent from Table 1 that the bodies elongate as the animals age. It should, however, also be noted (Table 1, Text-fig. 13) that in the rabbit and mouse, the length of the main chromaffin body (or cells derived therefrom) bears a constant relation to the length of the abdominal aorta, irrespective of age, and that the body ceases to elongate at the same time as the aorta. As indicated above, the extra-adrenal chromaffin bodies mature soon after birth and mitotic figures are few or absent in these structures throughout the greater part of the pre-pubertal growing phase of man, rabbit, guinea-pig and mouse. The change in the form of the chromaffin bodies is not, therefore, the result of a proliferation of phaeochromocytes. The present

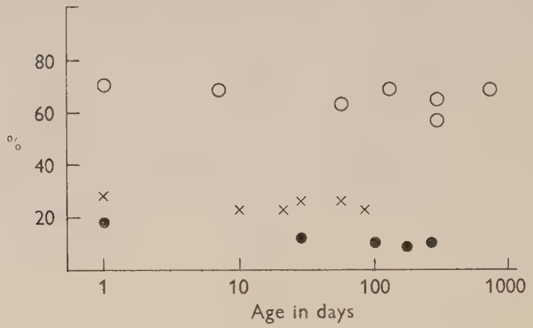
Table 1

Variation in length of aorta and main para-aortic chromaffin bodies with age

Animal	Age	Length of aorta* (mm.)	Length of P.A.B. (mm.)		P.A.B. as % length of aorta
Man	5 months	30·0	8·5		28
	18 months	27·0	11·0		40
	3 years	43·0	20·0		47
	5 years	52·0	32·0		61
	7 years	58·0	46·0		80
Rabbit	1 day	7·6	5·4		70
	7 days	16·0	10·8		68
	8 weeks	34·0	24·0		62
	16 weeks	61·0	42·0		68
	40 weeks	63·0	35·0		56
	40 weeks	70·0	43·0		62
	100 weeks	78·0	53·0		68
Mouse	1 day	3·3	0·9		28
	10 days	4·6	1·0		23
	21 days	6·4	1·4		23
	4 weeks	6·3	1·6		26
	8 weeks	12·0	3·1		26
	12 weeks	12·7	2·8		22
			P.A.B. Length (mm.)	P.A.B. Width (mm.)	
Guinea-pig	6·5 cm. foetus	8·7	1·4	1·7	16
	1 day	13·6	2·4	1·6	18
	4 weeks	21·0	2·5	3·1	12
	14 weeks	47·0	5·0	4·0	10
	24 weeks	40·0	3·6	3·3	9
	36 weeks	44·0	5·0	3·0	11

* Length of aorta measured from superior mesenteric artery to bifurcation.

findings are, however, entirely in keeping with this change being passive in type and due to traction exerted by the elongating nerve fibres. In both guinea-pig and man during the growing phase the length of the main chromaffin body bears a less constant relation to the length of the aorta. This difference could, however, be accounted for, in the case of the guinea-pig, by the close association of the body with the fibres of the renal plexus, which may result in the relatively marked increase in the transverse as well as the longitudinal dimensions of the organ (Table 1), while in man, the size and shape of the main body or bodies is so variable that no close correlation can be expected. It is, therefore, suggested that the differential growth of the sympathetic nerve fibres and chromaffin bodies results in mechanical forces which distort the bodies and result, in the rabbit, in an alignment of the phaeochromocytes with their long axis parallel to the aorta and pre-aortic nerve fibres (Coupland, 1956), while in the guinea-pig some chromaffin cells become aligned with their long axis parallel to the obliquely running fibres of the renal sympathetic plexus. It is, furthermore, suggested that in man and in the mouse, where the stroma of the bodies is minimal, this mechanical effect is sufficient to disrupt the structures completely, the cells being scattered in the pre-aortic region, whereas, in the rabbit and guinea-pig the supporting connective stroma is sufficiently abundant to prevent this dispersal.



Text-fig. 13. Length of the main para-aortic body of rabbit, mouse and guinea-pig, expressed as a percentage of the length of the aorta (from superior mesenteric artery to bifurcation) at different ages. Ordinate: percentage length of body. Abscissa: age in days (log scale). ●, rabbit; ×, mouse; ○, guinea-pig.

The fate of the scattered chromaffin cells which result from the disintegration of the main body in both man and mouse is difficult to assess, but, though it is more difficult to recognize isolated chromaffin cells than groups of these elements, it would seem that many do actually disappear.

The chromaffin cells of the smaller para-aortic bodies form circumscribed masses which are usually embedded in and completely surrounded by nervous elements. The adjacent nerve fibres are arranged irregularly. In consequence, the mechanical stresses which must result from the differential growth of the cells and nerve fibres, would be expected to have a less obvious effect on these structures than on the main bodies. The persistence of these elements with some slight modification is in accord with the above hypothesis.

In the mouse numerous vagal paraganglia, originally described by Goormaghtigh (1935), have been observed in association with fibres of the superior mesenteric plexus. The constituent cells are smaller than the chromaffin elements and do not give a positive chromaffin reaction. They persist in the adult animal and degenerative changes have not been observed in these cells.

Many collections of ectopic adrenal cortex have been observed in both the guinea-pig and mouse. In a 12-week-old female mouse one mass of ectopic adrenal cortex showed degenerative changes which were identical with those which affected the X-zone of the normal adrenal cortex. None of these ectopic collections of cells contained chromaffin elements.

SUMMARY

In the new-born guinea-pig and mouse a large discrete para-aortic chromaffin body lies in the pre-aortic region adjacent to the renal vessels.

In the guinea-pig the body alters in shape during the pre-pubertal phase of growth and the connective tissue stroma changes from a reticular to a collagenous fibrous type. A discrete healthy looking body still remains, however, in the 80-week-old specimen.

The main chromaffin body of the 3-week-old mouse disintegrates: some of the constituent phaeochromocytes degenerate, others persist and form small groups in the pre-aortic region. There is no evidence of a peripheral migration of chromaffin cells.

Cellular infiltration and widespread degeneration of the chromaffin bodies has not been observed and it is suggested that, in these animals as also in man and rabbit, the changes in the form of the main para-aortic bodies during the pre-pubertal phase of growth, are the result of mechanical forces; these forces being produced by the differential growth of sympathetic nerve fibres and contiguous chromaffin elements.

Small discrete chromaffin bodies exist in the pre-vertebral sympathetic ganglia of the guinea-pig, but have not been observed in the mouse. In both animals small non-encapsulated collections of chromaffin cells may be found in the ganglia of the lumbar para-vertebral sympathetic chain.

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EXPLANATION OF PLATES

Abbreviations: *A* = aorta; *P.A.B.* = para-aortic chromaffin body; *S.N.* = sympathetic neurones; *L.V.* = left renal vein.

Plate 1

Fig. 1. Transverse section through the main para-aortic body of a 6·5 cm. guinea-pig foetus. Formol-dichromate. Haematoxylin. (×35.)

Fig. 2. Transverse section through the rostral extremity of the main para-aortic body of a 6·5 cm. guinea-pig foetus. Formol-dichromate, haematoxylin. (×35.)

Fig. 3. Transverse section through the main para-aortic body of a 2-day-old guinea-pig. Formol-dichromate, haematoxylin. (×90.)

Fig. 4. Section through a small para-aortic chromaffin body lying in the coeliac ganglion of a 2-day-old guinea-pig. Formol-dichromate, haematoxylin. (×400).

Fig. 5. Chromaffin cells in the adrenal medulla of a 2-day-old guinea-pig. Formol-dichromate, haematoxylin. (×650.)

Fig. 6. Chromaffin cells of the main para-aortic body of a two-day-old guinea-pig. Formol-dichromate, haematoxylin. (×650).

Plate 2

Fig. 7. Transverse section through the main para-aortic body of a 24-week-old guinea-pig. Formol-dichromate, Giemsa. (×50.)

Fig. 8. Section through a small para-aortic body lying in the coeliac ganglion of a 24-week-old guinea-pig. Formol-dichromate, haematoxylin. (×200.)

Fig. 9. Transverse section through the main para-aortic body of an 80-week-old guinea-pig. Formol-dichromate, haematoxylin. (×90.)

Fig. 10. Chromaffin cells of the adrenal medulla of an 80-week-old guinea-pig. Formol-dichromate, haematoxylin. (×650.)

Fig. 11. Chromaffin cells of the main para-aortic body of an 80-week-old guinea-pig. Formol-dichromate, haematoxylin. (×650.)

Fig. 12. Transverse section through the main para-aortic body of a day-old mouse. Formol-dichromate, haematoxylin. (×120.)

Plate 3

Fig. 13. Transverse section through the rostral extremity of the main para-aortic body of a day-old mouse. Formol-dichromate, haematoxylin. (×120.)

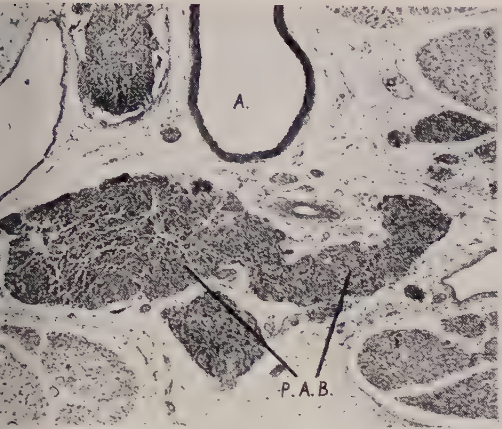
Fig. 14. Transverse section through the main para-aortic body of a 14-day-old mouse. Formol-dichromate, Giemsa. (×120.)

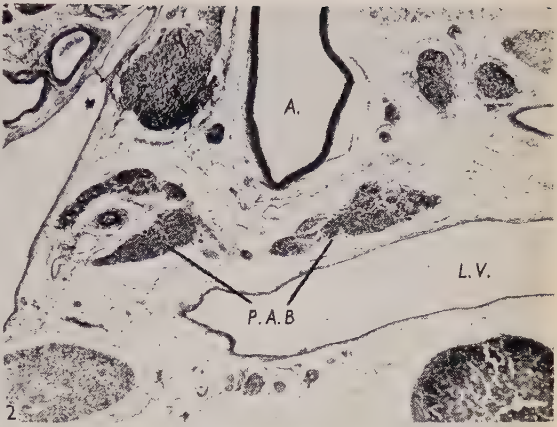
Fig. 15. Chromaffin cells of the main para-aortic body of a 14-day-old mouse. Formol-dichromate, Giemsa. (×500.)

Fig. 16. Transverse section through a 21-day-old mouse passing just caudal to the origin of the left renal artery. Formol-dichromate, haematoxylin. (×120.)

Fig. 17. Transverse section through a 4-week-old mouse passing just caudal to the origin of the left renal artery. Formol-dichromate, haematoxylin. (×120.)

Fig. 18. Chromaffin cells lying in the pre-aortic region of an 8-week-old mouse in the site originally occupied by the main para-aortic body. Formol-dichromate, Giemsa. (×540.)





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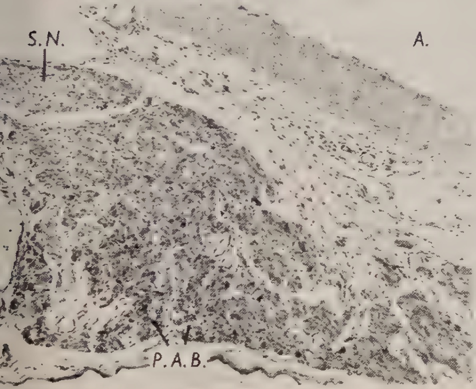

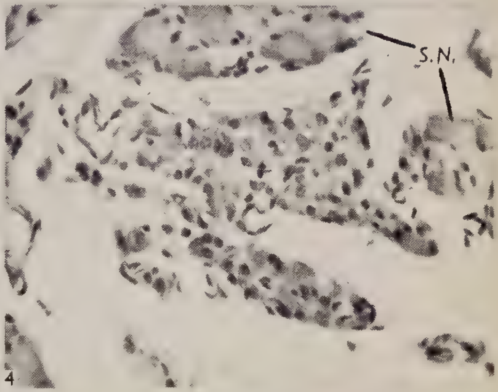

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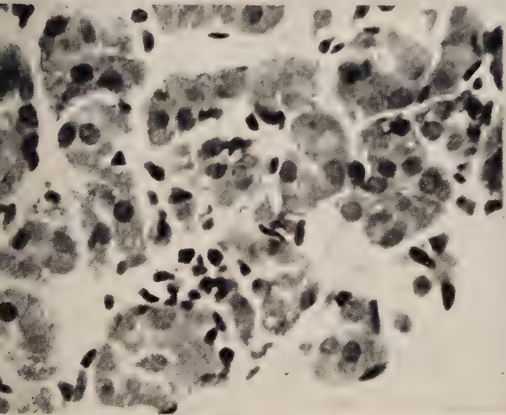

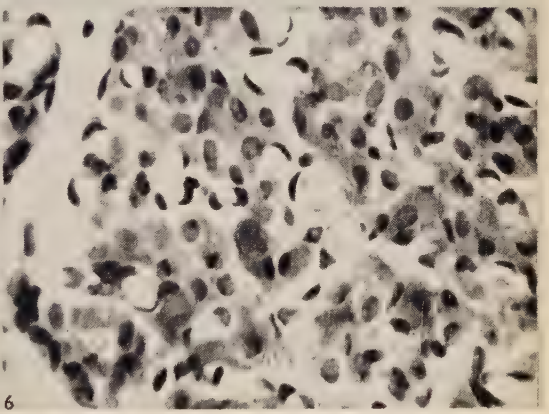

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COUPLAND—Post-natal distribution of the abdominal chromaffin tissue

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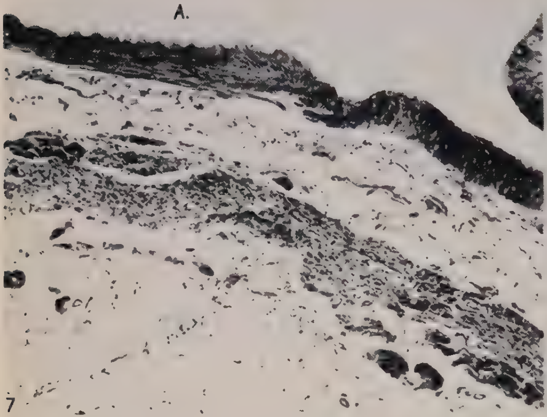
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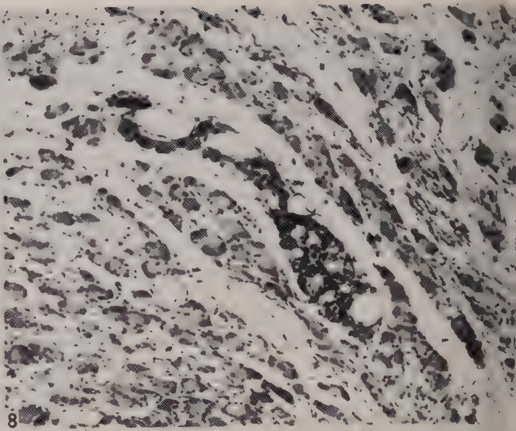
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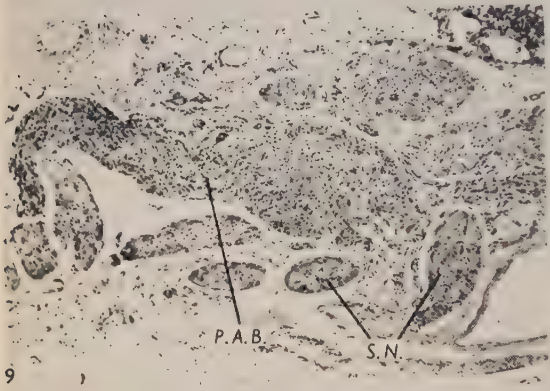
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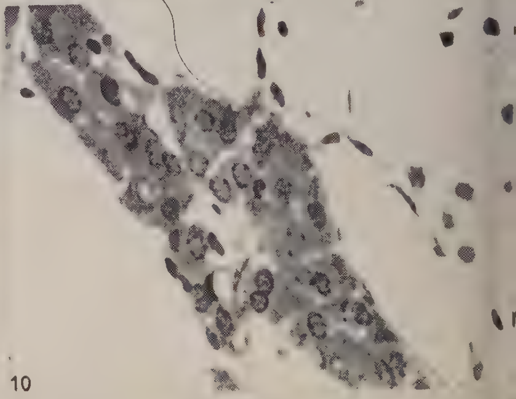
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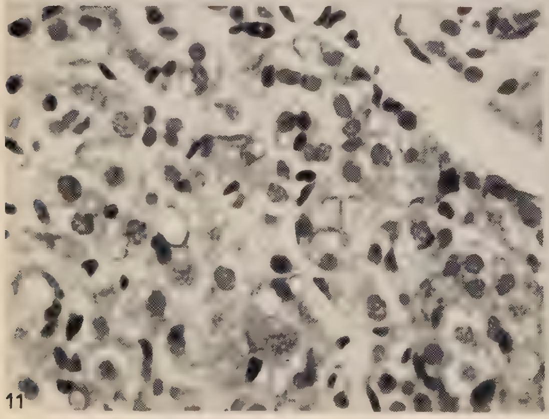
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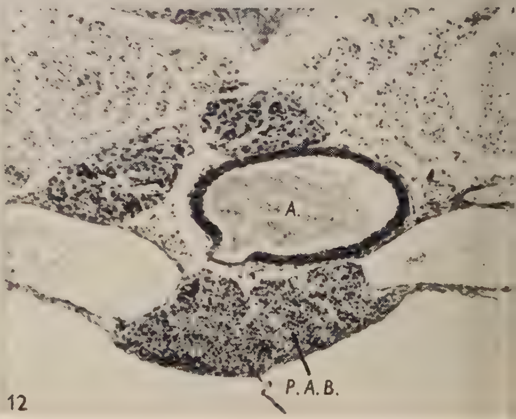
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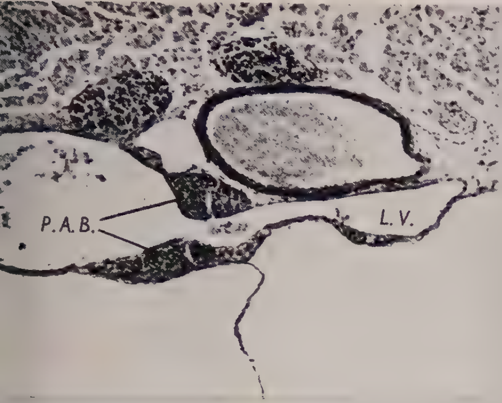
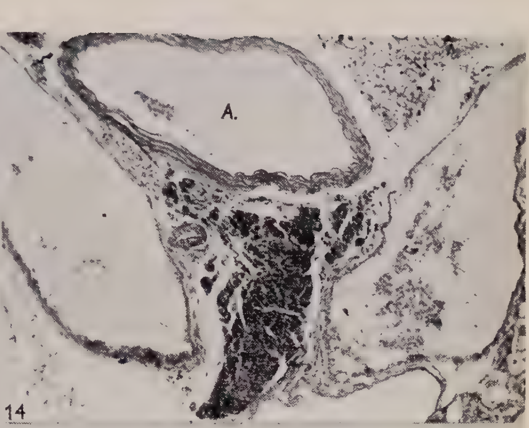
COUPLAND—Post-natal distribution of the abdominal chromaffin tissue





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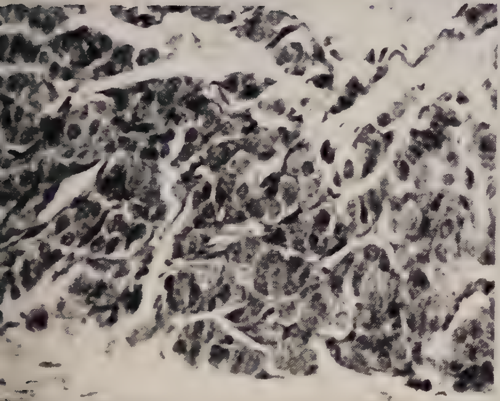

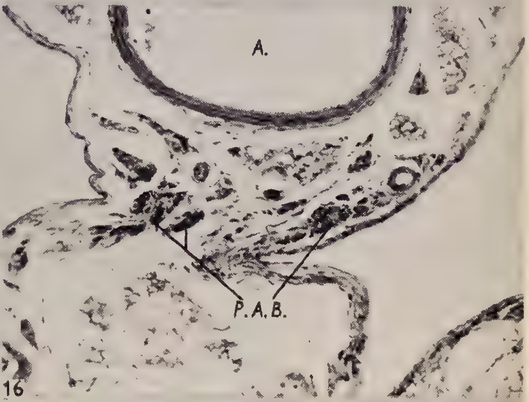

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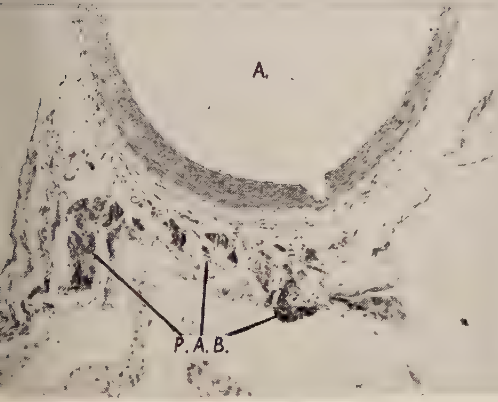

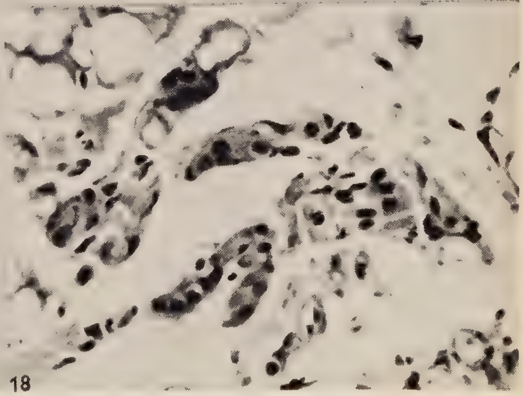

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COUPLAND—Post-natal distribution of the abdominal chromaffin tissue

THE BLOOD SUPPLY OF THE HUMAN PITUITARY GLAND

By J. P. STANFIELD

Department of Pathology, University of Liverpool

The blood supply of the human pituitary has been the subject of study by many workers in the last half century. This work has recently been summarized in two comprehensive papers by McConnell (1953) and Xuereb, Prichard & Daniel (1954), and the venous drainage has been described by Green (1957). These workers independently studied the pituitary blood supply by means of intra-arterial injections and serial sections. Though they are in fair agreement regarding the vascular anatomy, there are still one or two important points upon which they differ. The purpose of the present work is to reach a clearer understanding of the pituitary blood supply with particular regard to these points of variance.

The subject is of considerable importance at present in relation to three clinical conditions. (1) 'Spontaneous' ischaemic necrosis of the anterior lobe is a not uncommon complication of obstetrics, and occurs sometimes in other conditions. (2) During the last few years, many surgeons have transected the pituitary stalk with the object of destroying the blood supply of the anterior lobe. This operation always leaves part of the gland alive, the amount varying from case to case. (3) In surgical hypophysectomy, parts of the gland are usually left unintentionally *in situ*. Some of these parts undergo rapid ischaemic necrosis, but others survive.

MATERIALS AND METHODS

Twelve pituitary specimens were examined in the present study. Five consisted of the hypothalamus, the stalk and the pituitary gland, removed as a complete block of tissue with the surrounding soft tissue structures. These were used for the study of the external and internal vessels of the pituitary. The other seven specimens, used only for the study of the vessels inside the gland, had been removed from the skull in a routine manner and consisted only of the pituitary body and the lower half of the stalk.

Paraffin blocks of these specimens were cut serially at 20 μ, mostly in the transverse plane. Alternate sections were stained with haematoxylin and eosin and with Hart's modification of Weigert's elastic tissue stain. Each section was projected and drawn to allow a composite reconstruction of the course of the vessels.

SPECIAL DETAILS OF THE ANATOMY OF THE PITUITARY

The following account deals with certain fairly constant areas, which are closely related to the vascular pattern within the pituitary (Figs. 1, 2). Some of these areas are not clearly described in the literature.

The stalk

The pituitary stalk consists of a neural stalk and a pars tuberalis. The neural stalk is the column of nervous tissue extending from the hypothalamus to the posterior lobe of the pituitary body. The upper end of this column is shaped like the base of a cone and is applied over the nervous tissue in the floor of the infundibular recess of the third ventricle. Where the lower end enters the pituitary body it bends posteriorly to merge with the tissue of the upper pole of the posterior lobe; for convenience this bend within the pituitary body is called the 'genu' of the stalk, and corresponds to the 'intrapituitary stalk' of McConnell (1953) and the 'lower infundibular stem' of Xuereb *et al.* (1954). The region between the lower surface of the genu and the upper part of the anterior surface of the posterior lobe is called the 'subgenual angle'.

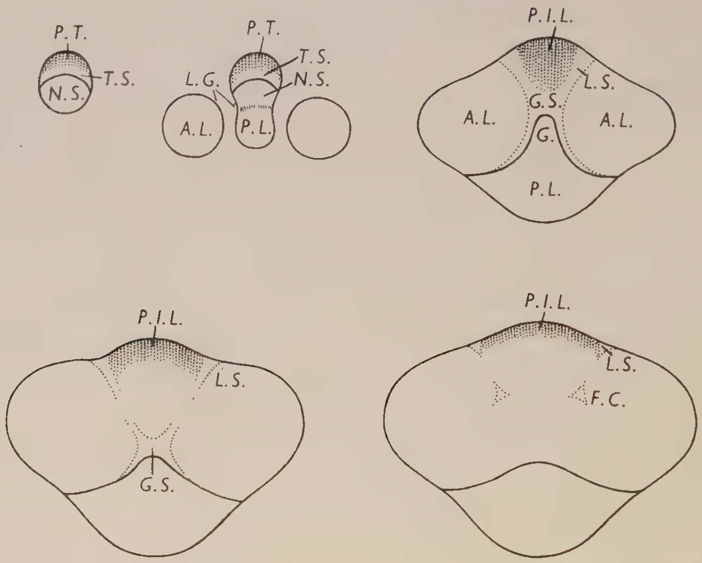

Fig. 1. Transverse sections of the stalk and upper part of the pituitary, at 1 mm. intervals.

KEY TO FIGS. 1 AND 2

A.L.	Anterior lobe	*N.S.*	Neural stalk
F.C.	Fibrous core	*P.I.L.*	Pars interloralis
G.	Genu	*P.L.*	Posterior lobe
G.S.	Genual septum	*P.T.*	Pars tuberalis
L.G.	Loral groove	*T.S.*	Tuberalis septum
L.S.	Loral septum		

The pars tuberalis is the layer of glandular acini over the anterior and lateral aspects of the neural stalk. It commences as a very thin discontinuous sheet in the recess between the anterior surface of the neural stalk and the posterior surface of the optic chiasma, and extends downwards in a thickening layer to the pituitary body where it merges with the anterior lobe in a manner to be described.

A layer of fibrovascular tissue is present in the posterior part of the pars tuberalis in the lower half of the stalk. This is called here the 'tuberalis septum'. It is thin and rather indefinite above, but increases to a thick layer towards the lower end of the stalk. It is sharply separated from the neural stalk posteriorly but less well defined from the pars tuberalis anteriorly.

The fibrovascular structure of the tuberalis septum continues into the pituitary body as a crescentic band over the front and sides of the genu of the neural stalk, and in this region it is referred to as the 'genual septum'. This lies between the genu and anterior lobe parenchyma; it contains all the portal vessels and a number of important arteries which will be described below. Below the subgenual angle the genual septum becomes continuous with the layer of epithelial cysts which separate the anterior and posterior lobes and which, in a human pituitary, represent the vestigial remains of the pars intermedia.

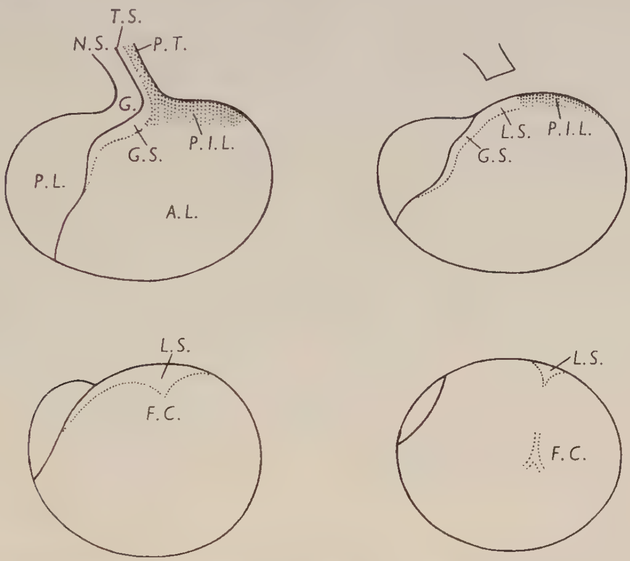

Fig. 2. Sagittal sections of stalk and pituitary at 1 mm. intervals from the midline.

The loral septa

The lateral parts of the superior surface of the anterior lobe project upwards above the level at which the stalk joins the body of the pituitary, so that there is a shallow gutter on each side of the insertion of the stalk. These extend forwards for some 2 or 3 mm over the superior surface of the anterior lobe, diverging as they go. These are described here as the 'loral grooves' because, at their anterior ends, the loral arteries enter the anterior lobe.

The loral grooves form the surface markings of two septa (the 'loral septa') which, in coronal sections of the lobe, have the shape of inverted wedges with their bases on the capsular surface, shallow in front but becoming deeper posteriorly, where they end by fusing with the lateral borders of the genual septum. They convey in their apices the fibrovascular sheath in which the loral arteries course backwards towards the base of the stalk. Anteriorly they are not very distinct structures, and are composed mainly of small acini of chromophobe cells, but posteriorly they become more fibrous.

Between the two loral grooves on the superior surface of the anterior lobe, the lower end of the pars tuberalis extends forward from the stalk as a tongue of glandular tissue, the 'pars interloralis'. This tongue is bounded laterally by the loral septa, but inferiorly it has no well-defined margin and merges with the main mass of the anterior lobe. It is composed mainly of chromophobe cells, resembling those found in the pars tuberalis, and numerous large sinuses traverse its substance.

The fibrous cores

In the centre of each lateral half of the anterior lobe there is a vertical column of fibrous tissue, 1½–2 mm. in height, which is referred to as the 'fibrous core'. It lies at about one-third of the distance from the midline to the lateral pole. Its vertical extent can most easily be expressed in the following way. If the anterior lobe is divided horizontally into four equal slices, the column of the fibrous core occupies the second of the slices from the top. Its upper extremity is continuous with the lower edge of the loral septum. The attachment may be at any point along the loral septum but is most commonly at its posterior end where it joins the lateral margin of the genual septum. Occasionally there may be two fibrous cores on each side, one arising from the anterior end of the loral septum and one from the more usual site at the posterior end.

The general shape of the fibrous core is like that of the trunk and roots of a tree. At its upper end it is narrow. As it passes downwards it becomes wider and finally it spreads out into a broad base from which processes of fibrous tissue branch out into the anterior lobe parenchyma for a short distance in all directions. In some cases a fibrous band runs transversely across from one fibrous core to its partner on the other side; this may happen at any part of the fibrous core.

In cross-section the fibrous cores are triangular and are composed of loose fibrous tissue containing an artery and several large sinusoidal vessels.

In the lower half of the anterior lobe there is no trace of any fibrous trabeculae.

THE ARTERIES OF THE PITUITARY

The course of the arteries in the whole series of pituitaries can be fitted into one comprehensive basic pattern (Fig. 3), though in any individual case only a certain proportion of the pattern is completed. The site of these vessels in serial transverse sections of the pituitary is shown in Fig. 4. In general the terminology of McConnell has been followed in the present description.

The superior hypophyseal arteries

These are constant vessels arising from the internal carotid artery just after it leaves the cavernous sinus. There are usually two on each side, an anterior and a lateral artery, and they approach the stalk about midway between the pituitary body and the hypothalamus. About 1 or 2 mm. before the anterior artery reaches the anterior surface of the stalk, it gives off the large loral artery which passes down in the subarachnoid space in front of and to the side of the stalk to enter the superior surface of the anterior lobe.

When the superior hypophyseal arteries reach the surface of the stalk they turn upwards and laterally, dividing within the subarachnoid space into a plexus of small arteries which surrounds the upper half of the stalk like a collar. Anteriorly this plexus extends from the midpoint of the stalk up to the apex of the infundibulo-optic recess. The plexus encircles the stalk, and posteriorly it extends up to cover the surface of the median eminence. It gives rise to numerous arteries which penetrate the stalk and median eminence.

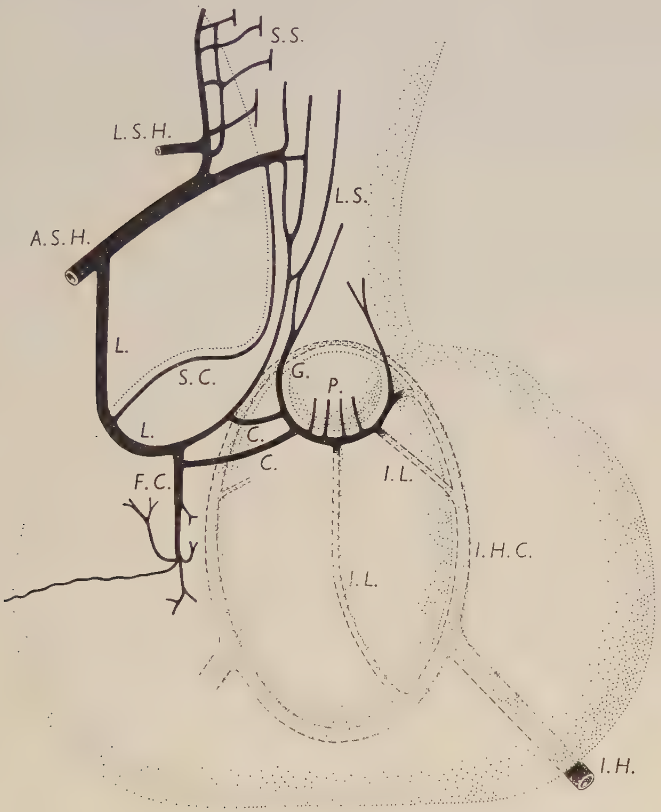

Fig. 3. Basic pattern of arteries of pituitary.

KEY TO FIGS. 3 AND 4

A.S.H.	Anterior superior hypophyseal artery	*L.*	Loral artery
C.	Communicating arteries	*L.S.*	Long stalk arteries
F.C.	Artery of fibrous core	*L.S.H.*	Lateral superior hypophyseal artery
G.	Genual artery	*P.*	Parallel vessels
I.H.	Inferior hypophyseal artery	*S.C.*	Subcapsular artery
I.H.C.	Inferior hypophyseal circle	*S.S.*	Short stalk arteries
I.L.	Interlobar arteries		

The short stalk arteries. These arise from the plexus of the superior hypophyseal arteries. After a short course into the neural stalk they turn upwards or downwards in the long axis of the stalk. The majority of these arteries terminate within the capillary complexes of the neural stalk.

The loral arteries. These two arteries arise from the anterior superior hypophyseal arteries and pass down in the subarachnoid space like a pair of reins to the anterior lobe. Each runs down about 2 mm. in front of, and about 2 mm. to the side of, the stalk, and it penetrates the superior surface of the anterior lobe at the anterior end of the loral groove about 2 mm. lateral to the midline. As it passes through the capsule of the anterior lobe, it gives off a branch, the 'subcapsular artery', which runs backwards in the loral groove. After the loral artery has entered the substance of the anterior lobe it passes backwards and inwards in the fibrous tissue in the deep margin of the loral septum to reach the lateral part of the genual septum. In this part of its course the artery gives rise to a downward branch, the 'artery to the fibrous core'. It then enters the genual septum and turns upwards in this structure towards the pituitary stalk. Within the septum it gives off an important but variable branch to the anterior surface of the genu which anastomoses with the interlobar branches of the inferior hypophyseal circle. The loral artery terminates by dividing into two or three 'long stalk arteries' which pass up the stalk in the tuberalis septum for a varying distance. These arteries will be described later.

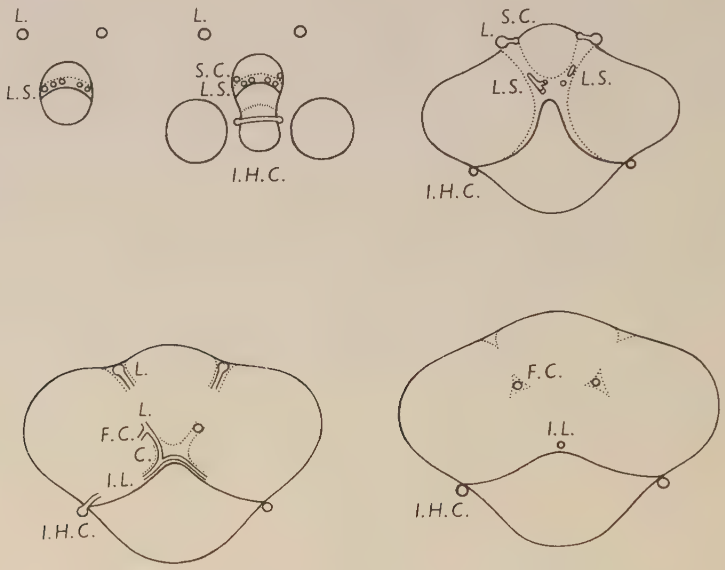

Fig. 4. Transverse sections of stalk and pituitary at 1 mm. intervals.

The subcapsular artery. This branch of the loral artery is variable and inconstant. It passes backwards just beneath the surface of the loral groove to the lower end of the stalk and thence turns upwards on the surface of the pars tuberalis to anastomose with a long descending branch of the superior hypophyseal artery plexus. It supplies branches to the pars tuberalis and the neural stalk.

The artery to the fibrous core. This artery arises from the loral artery as it enters the genual septum. The vessel enters the upper end of the fibrous core and passes down within its substance. At the lower end of the fibrous core it terminates in several small branches, arteriolar in size, which leave the fibrous core and are distributed to the parenchyma of the anterior lobe. They may be accompanied by a narrow sheath of fibrous tissue for a short distance. They have no obvious muscular coat, but can be identified by the fact that they have a thin internal elastic lamina. Some of them run out laterally and can occasionally be traced as far as the lateral pole or the inferior surface of the anterior lobe. Many of them are recurrent; after a short course downwards they turn upwards into the upper half of the anterior lobe and end as delicate arteriolar sinusoids.

The long stalk arteries. These arteries have already been mentioned as the terminal branches of the loral artery where it breaks up in the genual septum. There are usually two or three arteries on each side. They run upwards in the tuberalis septum or occasionally in the substance of the pars tuberalis. In the tuberalis septum, they often anastomose with branches of the interlobar arteries coming up from the inferior hypophyseal circle, which will be described below. As the long stalk arteries pass upwards, they give off the 'parallel vessels', which penetrate the genu and lower end of the neural stalk; these will also be described later.

About half way up the stalk, the long stalk arteries often receive large anastomoses from the superior hypophyseal trunks. From this point upwards they gradually penetrate the neural stalk and branch within it, sometimes anastomosing with other small arteries there.

Transverse anastomoses between the loral arteries or their branches on each side occur fairly frequently. The most common site is where the loral arteries turn upwards in the genual septum and divide into the long stalk arteries.

The inferior hypophyseal arteries

A single inferior hypophyseal artery arises on each side from the postero-medial wall of the first bend of the internal carotid artery in the cavernous sinus. Each artery approaches the lateral surface of the pituitary body, at about the junction of the middle and lower thirds of the groove between the anterior and posterior lobes, and divides on the surface into an ascending and descending branch. These branches run coronally in the interlobar groove and anastomose above and below with their partners of the opposite side, thus forming an arterial circle. At the upper end the anastomosis lies behind the stalk.

Three groups of vessels arise from the inferior hypophyseal circle.

(1) Direct penetrating branches to the posterior lobe. These have not been studied in detail in the present work.

(2) Capsular branches. The majority of these pass backwards in the capsular surface of the posterior lobe and supply penetrating branches into its substance. In addition there are a few small arteriolar branches from the lower part of the arterial circle which run forwards into the capsule over the inferior surface of the anterior lobe and terminate by penetrating a short distance into the parenchyma.

(3) The 'interlobar arteries'. These are large arteries which arise from the inferior hypophyseal circle and penetrate between the anterior and posterior lobes. There is usually one on each side and, though they may come from any point on the circle, the most common region is the upper third. They run medially between the two lobes to the region of the subgenual angle. During this course they give off many small branches, the majority of which penetrate and supply the posterior lobe, though an occasional twig runs forwards into the anterior lobe parenchyma. On

reaching the subgenual angle the interlobar arteries anastomose with each other in the midline and also give rise to two or three 'genual arteries'. These are small arteries which ascend on the anterior and lateral surfaces of the genu and supply the lower part of the genu with 'parallel vessels' which are described below. As mentioned earlier, these genual arteries usually anastomose with long stalk arteries.

From the interlobar artery near the anastomosis with its fellow, or sometimes from one of the lateral genual arteries, a relatively constant artery (the 'communicating artery') passes outwards and forwards in the lateral edge of the genual septum to the upper part of the fibrous core. Here it anastomoses either with the loral artery or with the artery to the fibrous core near its origin. The size of the communicating artery varies considerably. Sometimes it is larger than the loral artery and apparently contributes most of the blood supply to the fibrous core and the long stalk arteries; in other cases it is a small twig difficult to trace without an elastic tissue stain.

The parallel vessels. This is the name given by McConnell to the group of small arteries which cross the genu upwards and backwards, parallel to each other, and at right angles to its surface. They lie in well-defined septa. In the lower part of the genu they are supplied by the interlobar arteries from the inferior hypophyseal circle and by the genual arteries which branch from them. In the upper part of the genu they are supplied by the long stalk arteries or by the anastomoses between these and the genual arteries.

The capsular arteries

The inferior capsular artery which arises from the carotid artery in the cavernous sinus gives a supply to the parenchyma at the lateral pole of the anterior lobe. This supply is only by minute twigs, but can be important in special circumstances.

Variations

The preceding description of the arterial supply of the pituitary refers to the basic pattern constructed from a study of the individual arrangements encountered in this series. It is proposed to describe these variations briefly. They depend on the presence or absence of each individual artery or groups of arteries, and particularly the presence and size of the anastomoses between:

(1) the loral artery and the interlobar arteries via the communicating artery;

(2) the loral artery and the superior hypophyseal artery plexus via either the long stalk arteries, or the subcapsular artery;

(3) the interlobar artery and the long stalk arteries via the genual arteries.

If we consider each pituitary in this series of twelve as divided into two sides through a midline sagittal plane, there are twenty-four individual arrangements.

(*a*) In six cases the arrangement was essentially that of the basic pattern.

(*b*) The subcapsular artery was absent in seventeen cases.

(*c*) The loral artery was absent in four cases.

In two of these its place was taken by the subcapsular artery (Fig. 5).

In the third case its field of supply was taken over by a large interlobar artery; this gave rise to a large communicating artery which joined the artery to the fibrous core, and also anastomosed via the genual arteries with the long stalk arteries (Fig. 6).

In the fourth case the loral artery and the interlobar artery were both absent. The long stalk arteries arose from the superior hypophyseal arteries and descended the stalk to terminate in its lower end. The fibrous core artery was supplied by a branch from the lower part of the inferior hypophyseal circle which ran up in the substance of the anterior lobe and entered the lower end of the fibrous core (Fig. 7).

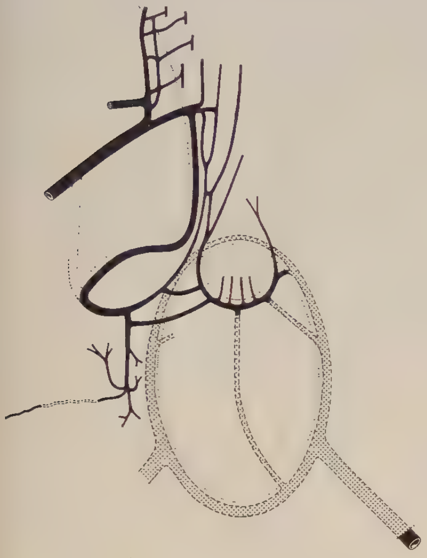

Fig. 5

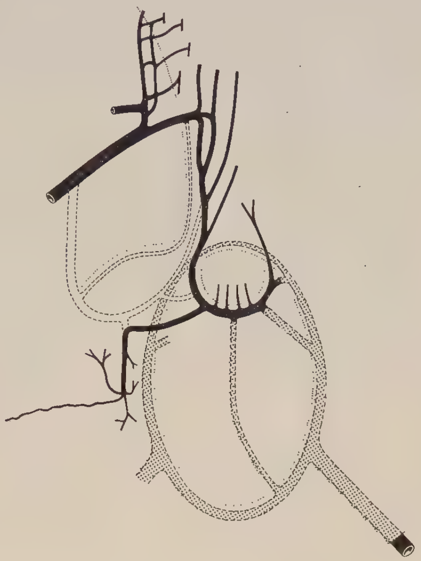

Fig. 6

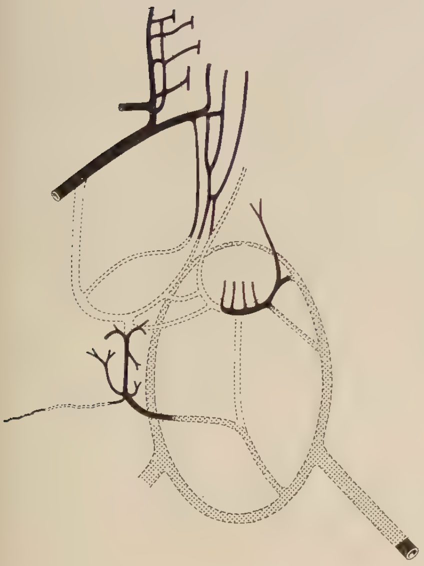

Fig. 7

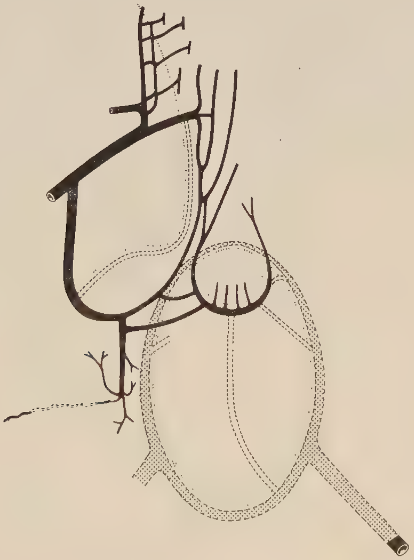

Fig. 8

Figs. 5–11. Variants on arterial pattern. Absent vessels indicated by dotted lines.

(*d*) The interlobar artery was absent or failed to reach the subgenual angle in four cases.

In three of these cases the genual arteries were supplied by the loral artery via the communicating artery (Fig. 8).

In the remaining case, they were supplied by the long stalk arteries and there was no artery to the fibrous core (Fig. 9).

(*e*) In four cases the long stalk arteries arose solely from the superior hypophyseal

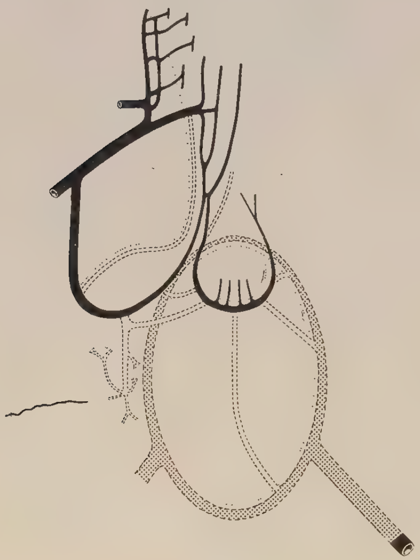

Fig. 9

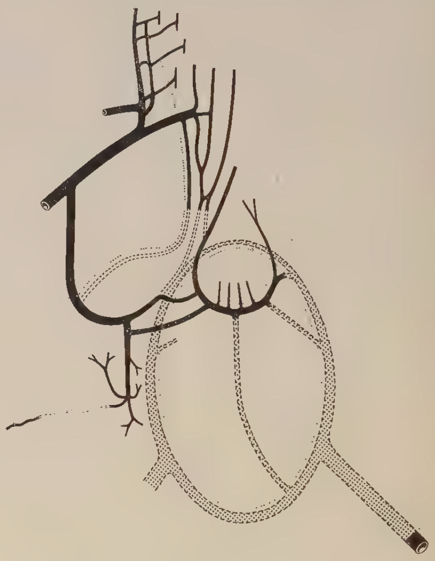

Fig. 10

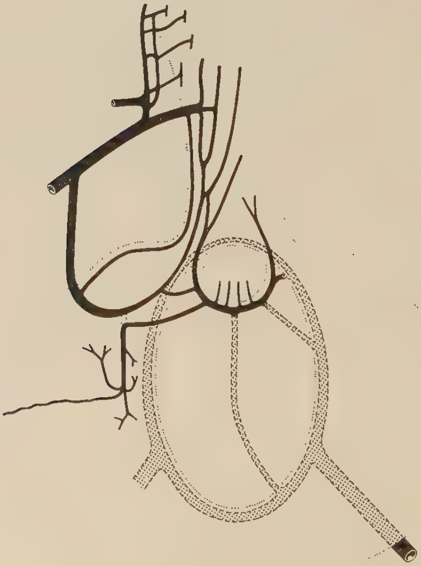

Fig. 11

artery plexus and, though they descended the stalk for a varying distance, they had no lower connexions. The loral artery in these cases supplied the fibrous core and anastomosed with the interlobar or genual arteries via the communicating artery, but it had no connexion with the long stalk arteries (Fig. 10).

(*f*) The artery to the fibrous core was absent or rudimentary in three cases.

(*g*) The communicating artery was absent or rudimentary in two cases.

(*h*) In one case the artery to the fibrous core was supplied only by the communicating artery and had no connexion with the loral artery (Fig. 11).

Transverse anastomoses, which have not been included in the above analysis, were present:

(i) between the loral arteries via the communicating artery in the subgenual angle in four cases (these represent the anastomoses which McConnell described as occurring at the lower ends of the long stalk arteries);

(ii) directly between the loral arteries whilst in the loral septa in one case;

(iii) between the loral artery on one side and the subcapsular artery on the other side in one case;

(iv) between the long stalk arteries at the lower end of the stalk in the tuberalis septum in one case;

(v) directly between the fibrous core arteries in one case.

Discussion of arterial supply

This description of the arteries of the pituitary is in broad agreement with those of McConnell and of Xuereb *et al.* There are, however, several features worth stressing.

The loral and long stalk arteries. Variation in the pattern of arteries from case to case accounts for many of the minor discrepancies between this study and those of McConnell and of Xuereb *et al.* There are, however, two points of difference which seem to be greater than could be accounted for in this way.

McConnell described the long stalk arteries as originating from the superior hypophyseal arteries where these penetrate the stalk, and as running downwards to unite and anastomose with the loral arteries in the genual septum. Xuereb *et al.* drew a more realistic picture of their course in describing these long stalk arteries as being the terminal ascending branches of the loral arteries. The findings in the present study show that this latter interpretation is applicable to the majority of cases.

The second point is that Xuereb *et al.* called the loral artery the 'artery to the trabecula' and described it, as had Fumagalli in 1941, as entering the distal end of the trabecula and running back along its length to the base of the stalk. If it be accepted that they use the word 'trabecula' to mean the structure described here as the loral septum, we agree with this part of their description. They stated that this artery within the trabecula was large and tortuous, and gave off no branches to the parenchyma, but that sometimes it had a small branch to the remainder of the fibrous tissue. This probably refers to the branch described here as the 'artery to the fibrous core'. It should, therefore, be emphasized that we consider this artery to be an important and very constant one; it runs down to the lower end of the fibrous core and ends there by branching into the parenchyma.

Anastomoses between the superior and inferior hypophyseal artery systems. Considerable arterial anastomoses occur in the region of the genual septum between the superior and inferior hypophyseal systems. Xuereb *et al.* stated that the anastomoses between the loral arteries and the interlobar branches of the inferior hypophyseal arteries were sufficient to provide a means by which arterial blood could be distributed to the stalk and to all the posterior lobe from either the superior or inferior hypophyseal arteries. McConnell found anastomoses between the long stalk arteries and the interlobar arteries, but considered that these were small and that the two systems were relatively independent apart from a small overlap in the genual region. In the present cases both sets of anastomoses were found: (*a*) between the loral arteries and the interlobar arteries in the region of the upper end of the fibrous core; (*b*) between the long stalk arteries and the interlobar arteries in the genual septum both medially and laterally. The size of these anastomoses varies considerably from case to case. Sometimes there is obviously a considerable overlap in the fields of supply of the two arterial systems, but in other pituitaries the anastomoses are scanty and quite small. The importance of this overlap has been demonstrated recently in experimental work on the rhesus monkey by Holmes & Zuckerman (1959).

End-arteries to the anterior lobe. McConnell said that there was some arterial supply to the anterior lobe from two sources: (*a*) the small branches from the fibrous core artery, and (*b*) a small artery to the lateral pole from the inferior capsular artery. Xuereb *et al.* state on the contrary that that there are no end-arteries to the anterior lobe.

The present study shows that there are undoubtedly end-arteries in the parenchyma of the anterior lobe, though they are small and little more than arterial sinusoids. Most of these arise from a single vessel, the artery to the fibrous core, which terminates in slender arterial twigs distributed throughout the upper half of the anterior lobe. Occasional small arteries penetrate the periphery of the anterior lobe from capsular arteries and from the interlobar branches of the inferior hypophyseal circle. In addition, the inferior capsular artery described by McConnell gives a small twig into the lateral pole of the gland and this may anastomose with a terminal twig from the fibrous core artery.

THE CAPILLARY STRUCTURES OF THE STALK

The short and long stalk arteries terminate by forming the central arteries of certain characteristic capillary structures which were described as 'gomitoli' by Fumagalli (1941).

Each gomitolo is about 1–2 mm. long and 50–100 μ wide. It consists of a central artery lying within a neuroglial sheath. Around the artery there is a coil of sinusoidal capillaries which are arranged spirally and longitudinally. At intervals the central artery gives off extremely short arterioles which open into the sinusoidal capillaries. These arterioles have no internal elastic lamina and are so short that they form little more than direct communications from the artery to the capillaries but they have a strong muscular sphincter around them. In this way the central artery becomes distributed to the sinusoidal capillaries and usually terminates at the end of its own gomitolo. Occasionally it continues on as a smaller vessel into the tissue beyond.

The capillaries of the gomitolo form a plexus, with many intercommunications. At various points on the outer part of the gomitolo, capillaries emerge and run out into the substance of the neural stalk where they join the 'pampiniform network' which is described below.

The gomitoli are found in the upper half of the neural stalk and in the median eminence. In the neural stalk they lie longitudinally and occur in all parts of the tissue. In the median eminence they are directed vertically upwards from the pial surface; they extend to within a short distance of the floor of the third ventricle, but do not reach it. The gomitoli here occupy a wedge of tissue adjoining the upper part of the neural stalk; as this wedge continues backwards, it becomes narrower and more restricted to the midline and finally ceases abruptly. There is a sharp margin between the tissue which contains the gomitoli and the neighbouring brain substance, whose capillary structure is similar to that of the rest of the brain. There are no significant vascular communications between these two parts.

The gomitoli were first described by Tello in 1912 and have been identified since then by all workers who have studied the vascular anatomy of the pituitary. Most of the literature has been reviewed by McConnell and Xuereb *et al.*

The function of these structures is not yet established, but they certainly appear to act as a mechanism controlling the flow of arterial blood into the pampiniform network of the stalk and thence into the portal vessels.

The pampiniform network. This term was introduced by McConnell to describe the network of capillaries throughout most of the neural stalk. In the upper part of the stalk it is not very prominent, but further down it becomes greater in amount because of the addition to it of more and more emergent capillaries from the gomitoli.

The capillaries draining the pampiniform network of the upper half of the stalk congregate to a number of longitudinal fibro-vascular septa which penetrate the neural stalk for a short distance from its surface. By way of these septa the capillaries reach the surface of the neural stalk and join together to form small surface sinuses. At the front of the lower third of the neural stalk and the genu, there are three or four large and constant vascular septa. These form a pathway for the sinusoids of the pampiniform network of the lower stalk and genu to the surface sinuses, and they also convey the arterial parallel vessels from the arteries in the genual septum inwards to the centre of the genu and lower neural stalk.

THE PORTAL VESSELS

The portal sinuses which have arisen in this way from the pampiniform capillary network run down along the surface of the neural stalk. They are arranged fairly constantly in anterior and posterior groups, which are named according to their relation to the neural stalk (Fig. 12).

The posterior portal sinuses

These commence on the inferior surface of the median eminence as two or three small sinuses. They descend the posterior surface of the neural stalk, branching and reuniting with each other, and draining other small sinuses which reach the surface from the pampiniform network. In the lower third of the stalk they have collected into five or six large vessels up to 200 μ in diameter, arranged round the posterior two-thirds of its circumference. These large sinuses have some muscle in their wall. They run a fairly straight course downwards on the posterior surface of the stalk, occasionally dipping into its substance for a short distance. Near the lower end of the stalk they incline forwards along the postero-lateral surface of the neural stalk, and form a group on each side at the point where the stalk joins the body of the pituitary. Immediately below this they lose their muscle coat, and fan out abruptly into the lateral parts of the anterior lobe, in general being distributed according to their original positions, those more anteriorly passing to the antero-lateral parts of the anterior lobe, and those posteriorly to the postero-lateral parts. One or two sinuses on each side do not turn outwards with the rest of the group, but course downwards into the centre of the anterior lobe and finally spread out laterally as they approach the inferior surface.

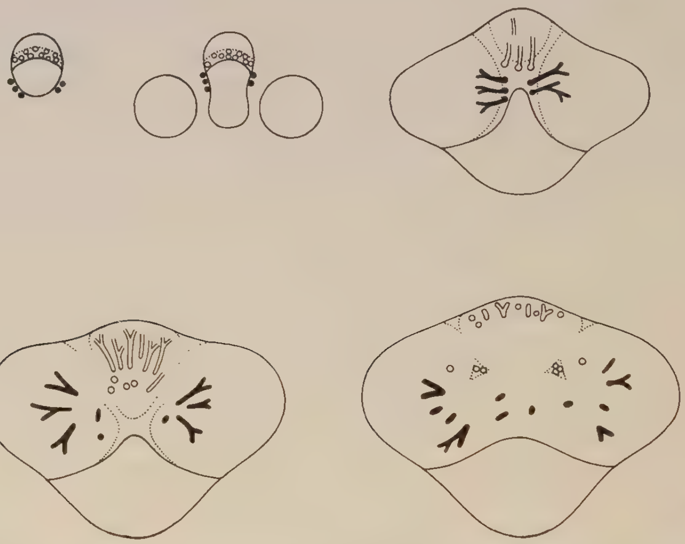

Fig. 12. Transverse sections of stalk and pituitary at 1 mm. intervals. Anterior portal sinuses in outline; posterior portal sinuses black.

The anterior portal sinuses

These sinuses have a very much shorter course than the posterior. They gather in the upper part of the stalk as a layer of small sinuses in the tenuous upper end of the tuberalis septum between the antero-lateral surface of the neural stalk and the glandular acini of the thin pars tuberalis. Their number is continually augmented by sinusoids reaching the surface of the neural stalk by way of the vascular septa, and they form the majority of the vessels in the main part of the tuberalis septum. In the lower part of the neural stalk and the upper part of the genu, further portal sinuses of this group are formed from sinusoids which accompany the parallel vessels and drain the capillary pampiniform network of these regions.

The anterior sinuses are much more numerous than the posterior; they number about twenty to thirty at the junction of the stalk with the pituitary. In addition, they tend to be smaller in calibre than the posterior sinuses; their walls are thicker and more muscular, and often contain a number of elastic fibres.

The anterior portal sinuses are distributed in three ways:

(1) The majority turn forwards at the lower end of the stalk, and run in a broadening band in the pars interloralis beneath the surface of the anterior lobe. One by one they turn sharply downwards into the substance of the anterior lobe and are distributed to its superior and anterior parts.

(2) Four or five of the anterior portal sinuses which lie at the lateral margins of the tuberalis septum turn sideways to accompany the posterior sinuses into the antero-lateral parts of the anterior lobe.

(3) A few of the most posterior sinuses of the group run down into the genual septum and receive small branches draining the genu of the stalk. Some of them continue downwards and are distributed in the posterior part of the anterior lobe. An important group enter the fibrous core on each side and divide into a number of wide thin-walled sinusoids which run downwards with the artery to the fibrous core. They are eventually distributed into the anterior lobe in the slender fibrous processes which radiate out at intervals along the whole length of the fibrous core. These fibrous core sinusoids are remarkable in that the calibre of each individual vessel appears to be very much larger than the anterior portal sinuses from which they undoubtedly arise.

The portal vessels were first recognized independently by Popa & Fielding (1930), and by Pietsch (1930). These vessels were thought at first to carry blood from the sinusoids of the anterior lobe into the capillaries of the hypothalamus (Popa & Fielding, 1930; Collin & Fontaine, 1936) or into the capillary complexes of the stalk (Fumagalli, 1941). Further studies by many workers have shown, however, that these vessels constitute the outflow for the extensive capillary formations in the median eminence and the stalk, and provide the main blood supply to the anterior lobe (Wislocki & King, 1936; Wislocki, 1938; Morin, 1940; Green & Harris, 1947; Green, 1951; McConnell, 1953; Xuereb *et al.* 1954). Some recent German workers have suggested on the basis of injection preparations and histology that the direction of flow within the portal vessels might change in accordance with pressure differences in the anterior lobe and stalk (Romeis, 1940; Pfeifer, 1951; Spanner, 1952), but this seems improbable on anatomical grounds.

McConnell described the anterior portal sinuses and two larger posterior sinuses arising from the capillaries in the median eminence and upper part of the stalk and passing down its whole length. The two posterior vessels turned forwards around the lower end of the stalk to join the remaining anterior and lateral sinuses in the small wedge of anterior lobe tissue lying in front of the lower part of the stalk.

Xuereb *et al.* divided the portal vessels according to the length of their course; they particularly mentioned the short portal sinuses arising in the genual septum from the capillaries of the lower end of the stalk and the genu, and said that these portal sinuses were distributed in the posterior part of the anterior lobe.

SUMMARY

1. A study has been made of the blood supply of the human pituitary by means of thin serial sections.

2. Certain anatomical structures are named to facilitate the description of the intrapituitary vascular arrangements.

3. The variations encountered in the course of the arteries within the pituitary can be included in a comprehensive basic pattern. This pattern is in general agreement with the recent findings of McConnell (1953) and Xuereb *et al.* (1954) though there are certain points of disagreement.

Anastomoses between the superior and inferior hypophyseal artery systems occur in the fibrovascular region in front of, and lateral to, the intrapituitary part of the neural stalk. These anastomoses are very variable in number and size, and it is not possible to generalize regarding the degree of overlap of the territories supplied by the two systems.

A variable number of small end-arteries supply the anterior lobe directly with arterial blood. The largest and most constant of these is the artery to the fibrous core, but small arteries penetrate the anterior lobe from the capsule and the interlobar region. Their terminal arteriolar twigs are only distinguishable from the anterior lobe capillary sinusoids by means of good elastic tissue staining.

4. The coiled capillary structures or 'gomitoli' in the upper half of the neural stalk have a central artery from which very short muscular arterioles open almost immediately into the sinusoidal capillaries. The appearances suggest that the gomitoli control the flow of blood into the sinusoids of the stalk.

5. The capillaries of the gomitoli become distributed into a network of sinusoids throughout the whole neural stalk. These sinusoids run finally to the surface of the neural stalk where they drain into the portal sinuses.

6. The portal sinuses form the sole means of drainage of the capillary structures of the stalk. They are arranged in anterior and posterior groups according to their relation to the neural stalk; the few large posterior sinuses pursue a long course from the median eminence to the anterior lobe, while the anterior sinuses are much more numerous and shorter. They are distributed to the anterior lobe.

7. The anterior lobe receives its blood supply from two sources: the hypophyseal portal system and the special arteries described in this paper.

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BONE-MARROW VOLUME IN CATS

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Bone-marrow volume has recently been estimated in guinea-pigs both under normal conditions and in conditions of erythropoietic stimulation (Hudson, 1958*a*, *b*). These data, obtained by the improved agar method, were found of considerable value in studies of cellular equilibria in blood and blood-forming tissues carried out by Yoffey (1957) and Yoffey, Hanks & Kelly (1958). No measurements of bone-marrow volume appear to have been made in the cat, although the cellular constituents of the marrow of this species have often been the subject of study. [The investigations of Sawitsky & Meyer (1947) and Lawrence, Syverton, Shaw & Smith (1940) may be cited in example.] It appeared that such measurements would not only be valuable for comparison with those already obtained in guinea-pigs but might also be of importance in future studies of feline bone marrow.

MATERIALS AND METHODS

The cadavers of apparently full-grown mongrel cats of both sexes were used in the study. The ages of the animals were unknown. All were well nourished and showed no evidence of disease. In three of the animals studied, many of the epiphyses were subsequently found to be un-united, although in each case the epiphysis at the lower end of the humerus had fused with the diaphysis.

The procedures used for macerating the skeleton and for estimating bone-marrow volume were essentially those already described by Hudson (1958*a*). Only a brief outline of the method will therefore be given, noting the features peculiar to the cat.

The cadaver was skinned and eviscerated. It was then macerated in various sections: skull, trunk (excluding tail), tail, limbs of one side. In an attempt to segregate the bones containing ‘red’ marrow from those containing ‘fatty’ marrow, the limbs were further subdivided on the basis of the appearances shown by the marrow of the opposite side. (In the pes and manus and usually in the lower part of tibia, radius and ulna, the marrow was very pale and showed little or no haemopoietic activity on histological section; this marrow was considered to be ‘fatty’. There was, however, no sharp line of junction.) In each case, the great long-bones were transected to facilitate maceration. When maceration (consisting of proteolytic digestion, washing and de-fatting) was complete, the frontal and sphenoid bones together with the mandible were transected in order to permit the subsequent removal of agar from the large air-sinuses and mandibular canal. The ethmoid was excluded from the subsequent procedures, in view of the impracticability of removing agar from its labyrinthine spaces by mechanical means. The volume of bone substance in each part of the skeleton was measured, using air-free distilled water at 16° C; a density bottle was used with a neck diameter of 4·8 cm., and a capacity of 250 ml. The bones were then boiled in a saturated solution of agar for 15 min. and the agar allowed to set, thus filling all the spaces in the interior. After the bones had been removed from the solid agar (the agar in the medullary cavities of the long-bones being cut flush with the sawn ends), they were washed and surface-dried, care being taken to exclude agar from such spaces as the tooth sockets, air sinuses, the grooves between the ungual crest and process of the terminal phalanges, etc. The total volume of each part of the skeleton was then measured. The difference between the two volumes represented the volume of agar taken up and this, when corrected for the volume of agar present in bone spaces which would not normally contain marrow, was a measure of the bone-marrow volume. (This correction was based upon the agar-uptake of pieces of marrow-free bone obtained from the same skeleton.) In this way, the volumes of both total and 'red' marrow could be calculated, assuming for the latter purpose that 'fatty' marrow was confined to the tail and the distal part of the limbs.

RESULTS

The total volume of bone marrow was estimated in twelve animals, the body weights of which lay within the range 2·1–3·95 kg. The mean volume of bone marrow was 54·2 ml. (36·1–72·1 ml.). The standard deviation was 10·2 ml. The marrow volume thus represented approximately 1·9% of the body weight (1·3–2·4%). The results are recorded in Table 1.

The relative distribution of the total marrow volume between skull, trunk and limb bones was as follows: skull (excluding ethmoid) 11·4 % (7·3–13·4 %); trunk (including tail) 34·0 % (30·2–39·8 %); limbs (including girdle bones) 54·6 % (48·9–60·6 %). The corresponding figure for the caudal vertebrae alone was 2·4 % (1·7–3·0 %). The volume of 'red' marrow was estimated to constitute a mean of 86 % (82–91 %) of the total marrow volume.

Table 1. *Bone-marrow volume data for twelve cats*

Sex	Body weight (g.)	Bone-marrow volume (ml.)
M.	3950	65·7
M.	3400	67·5
F.	3350	51·2
F.	3300	72·1
M.	3150	52·2
F.	2900	49·3
M.	2800	59·6
F.	2700	36·1
F.	2500	47·0
F.*	2350	47·9
F.*	2300	52·3
M.*	2100	50·0

* In these experiments, many of the epiphyses were un-united.

The mean weight of the macerated skeleton was 137·3 g. (89·5–206·2 g). The relative distribution of this weight between skull, trunk and limb bones was as follows: skull (including ethmoid) 17·0 % (11·4–22·0 %); trunk (including tail) 27·1 % (23·7–30·0 %); limbs (including girdle bones) 55·9 % (53·4–58·6 %). The mean liver weight was 91·8 g. (58·1–146·9 g.).

DISCUSSION

Total volume of bone marrow

The mean value for bone-marrow volume in this series of normal cats was 54·2 ml., representing approximately 1·9 % of the body weight. If one considers only the nine animals in which union of the epiphyses was complete, the values for adult cats may be taken as 55·6 ml. and 1·8 % of the body weight. The latter figure may be compared with similar figures obtained in adult animals of other species. In adult rabbits, Dietz (1944) obtained a figure of 2·2 %, whilst the figure for adult guinea-pigs was approximately 1 % (Hudson, 1958*a*). Garcia (1957), in the course of radioiron time-distribution studies, calculated that the marrow of the 250-day rat constituted 0·8 % of the body weight. These figures stand in marked contrast to the mean value of 4·6 % obtained by Mechanik (1926) in adult man, although the fact that his observations were not made on normal material needs to be borne in mind. It would appear important that further bone-marrow volume measurements should be carried out in normal adult man and in adults of other species, in order to ascertain whether a relatively large amount of bone marrow is a characteristic feature of larger animals.

Measurements of total marrow volume have also been carried out by other investigators, but in view of the very small number of observations in each series, they will not be discussed here.

A study of the distribution of total marrow volume between skull, trunk and limb bones showed that the skull of the cat contained a strikingly smaller proportion of marrow than did the skull of the guinea-pig—by contrast, the limb bones contained relatively more marrow. It would appear from Table 2 that these differences may be related to similar differences in the proportions of the skull and limb bones themselves. The differences mentioned were significant at the 0·01 level of probability.

The volume of 'red' marrow

The volume of 'red' marrow (i.e. marrow showing haemopoietic activity) was estimated as constituting a mean of 86 % of the total marrow volume, but this figure can only be regarded as very approximate. There was, for example, no sharp dividing line between 'red' and 'fatty' marrow in the limb bones, and the 'red' marrow of the femur and humerus had such a high content of fat that it floated when placed in water. Histological sections of the marrow plug of the femur did, however, confirm the presence of considerable haemopoietic activity in this situation, in contrast to the marrow of the pes, which appeared to have little or none. The assumption that the bones of skull and trunk (except tail) contain 'red' marrow, rests mainly on findings in other species, although some observations were made in cats by Varićak (1938). It is noteworthy that the mean figure of 86 % accords well with that of 85–90 % given by Dietz (1944) for adult rabbits and that of 89 % obtained in the 400 g. guinea-pig. Mechanik (1926) obtained a corresponding figure of 50 % for the human and it would appear that the human has a much larger reserve of 'fatty' marrow that the other species here considered.

In order to emphasize the relatively large size of the 'red' marrow, considered as an organ, it is customary to compare its weight (or volume) with the liver weight. In the present observations, a mean value of approximately one half was obtained.

The range of individual variation

One of the striking features of this series was the very large range of individual variation. For example, the bone-marrow volumes of the cats with united epiphyses ranged from 36·1 to 72·1 ml., whilst the body weights varied from 2·5 to 3·95 kg., and the skeleton weights from 103·5 to 202·2 g. Such variations must inevitably complicate quantitative studies of haemopoiesis carried out, for example, in veterinary practice. In our laboratory these difficulties are obviated by using, for such studies, animals of a closely inbred strain and the same approximate age and body weight.

Table 2. *The anatomical distribution of bone marrow and bone substance*

Percentage of total marrow volume in:	Guinea-pig (Hudson, 1958*a*) (%)	Cat (%)
Skull	19·5	11·4
Trunk	31·5	34·0
Limbs	48·9	54·6
Percentage of total skeleton weight in:		
Skull	37·5	17·0
Trunk	24·8	27·1
Limbs	37·8	55·9

SUMMARY

1. Bone-marrow volume was measured in twelve normal cats of 2·1–3·95 kg. body weight, employing the improved agar method.

2. The mean value was 54·2 ml., representing 1·9 % of the body weight. Considering only the nine animals which had complete union of the epiphyses, the corresponding figures were 55·6 ml. and 1·8 %.

3. Of the total marrow, a mean of 11 % was found in the skull, 34 % in the trunk and tail, and 55 % in the limb and girdle bones.

4. It was estimated that haemopoietic activity was present in approximately 86 % of the total marrow.

5. The range of individual variation between normal adult animals was noteworthy.

I wish to express my gratitude to Prof. J. M. Yoffey for his constant interest and encouragement; to Mr R. Pollock of the Glass-shop in the Department of Physics for making the special density bottle; to Mrs M. Galvin for her help in preparing the manuscript.

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REVIEWS

The Developmental Biology Conference Series, 1956.
Paul Weiss, Organizer and General Chairman.

Regeneration in Vertebrates. Ed. by C. S. Thornton. (Pp. xi + 108, 24*s.*) 1959.

Embryonic Nutrition. Ed. by Dorothea Rudnik. (Pp. xi + 113, 24*s.*) 1958.

Dynamics of Proliferating Tissues. Ed. by Dorothy Price. (Pp. xv + 95, 24*s.*) 1959.

Cytodifferentiation. Ed. By Dorothea Rudnik. (Pp. xi + 148, 28*s.*) 1958.

Endocrines in Development. Ed. by R. L. Watterson. (Pp. xi + 142, 30*s.*) 1959.

Environmental Influences in Prenatal Development. Ed. by Beatrice Mintz. (Pp. xiii + 87, 22*s.* 6*d.*) 1958.

Immunology and Development. Ed. by M. V. Edds. (Pp. xi + 59, 19*s.*) 1958. Chicago: University of Chicago Press, London: Cambridge University Press.

These seven slim volumes are reports of symposia on selected aspects of developmental biology which were held under the auspices of the National Academy of Science and the National Research Council in Universities and Research Centres in the United States in 1956. The Conference Series brought together nearly 300 American and fifty-four scientists from nineteen other countries 'less for a display of most recent technical advances than for a concerted examination and evaluation of the contributions of their various specialities to the elucidation of focal issues of developmental biology'. As is inevitable in such a project there is an absence of uniformity in the individual presentations and from volume to volume. In his general preface to the series Dr Paul Weiss explains that this lack reflects the informal spirit of the meetings and accents the main theme of the Conference: that developmental biology is currently in a state of flux, fitting no rigid mould and shaping its course as it gains momentum by the growth and confluence of many tributaries. He adds the hope that the publication of the series will add to that momentum. There can be little doubt that it will do so.

The individual volumes are attractively produced and have been well edited.

J. D. BOYD

Sympathectomy: An Anatomical and Physiological Study with Clinical Applications. By P. A. G. Monro. (Pp. xx + 290; 84 illustrations; 75*s.*) Oxford University Press.

Progress in pharmacology is continuous, sometimes spectacular, and it may be that future generations will remember the operation of sympathectomy as a form of treatment which, *faute de mieux*, held the stage for a few uneasy decades. That the author of this excellent monograph cherishes this hope there can be no doubt, nor can it be in question that what he has written may well have speeded the day. However that day is not yet, and were this book to contain nothing more than the counsel offered to surgeons faced with deciding the type of sympathectomy operation to be performed it would still have been well worth writing. But Dr Monro has done very much more than that, and the reviewer is confident that any one, laboratory worker or clinician, to whom the sympathetic nervous system continues to offer a challenge will find his interest quickened and his understanding deepened by a close study of this work. But close it will have to be, for though well written this is not an easy book to read. Nor in the nature of things could it be, for the system dealt with is far from simple and the author has not fought shy of any of its complexities.

Although sympathectomy operations have been performed for more than half a century scant attention has been paid to their long-term effects on the function and structure of the sympathetic nervous system itself. Dr Monro has now remedied this state of affairs and his records of continued observations made on more than fifty patients over periods of years reveal changes in sympathetic activity which occur at varying and repeated intervals after operation. His methods for detecting sudomotor and vasomotor activity are clearly described, and details of the apparatus used are given in full in a valuable appendix.

Amongst the problems encountered by investigators in this field is that of retention of sympathetic activity, as evidenced by sweating, in areas which might be considered—certainly on first thought—to have been denervated by sympathectomy. For example, dermatomes T. 12–L. 3 and S. 4 and S. 5 retain sudomotor activity following one type of conventional operation, as also does the central mask of the face following another. In seeking explanations for these facts the author first marshals the evidence of anatomy, embryology, physiology and pharmacology with great thoroughness, and then as the data permit either provides firm answers or offers very sound suggestions. While certain of his suggestions are avowedly conjectural the steps in his reasoning are always logical and difficult to fault.

Recovery of function after sympathectomy has long perplexed critical investigators. For several reasons explanations which invoke regeneration of pre- or post-ganglionic fibres are inadequate in many cases, and the points adduced by the author in favour of a reorganization of preganglionic fibres by collateral sprouting are well chosen.

Much of this volume is a record of personal observation made over a period of 10 years, and as such could well stand alone. At no place however does this in fact happen; always are the author's own noted facts set against the historical background side by side with those of present authorities. And indeed many of the papers quoted are scarcely months old.

The reviewer would wish to end by offering his sincere congratulations to Dr Monro on a fine piece of scholarship which will occupy a high place in the literature on the sympathetic nervous system.

E. W. WALLS

Textbook of Comparative Histology. By WARREN ANDREW, Ph.D., M.D. (Pp. xix + 652; 220 text-figures and 4 plates; £6. 0*s.* 0*d.*) New York: Oxford University Press. 1959.

In the past, the student interested in comparative histology has had to obtain information either from some of the older and more detailed text-books of comparative histology which were not always readily available or from scattered articles dealing with histology in journals of anatomy and zoology. Dr Warren Andrew has brought together much of this information in a format suitable for the undergraduate student.

In scope the text-book covers all aspects of animal life and the author justifies this scope of subject-matter by explaining that the very breadth of the field of biology forms one of its most appealing features. The first two chapters or approximately one-twelfth of the book are devoted to a general consideration of cells and tissues. After that the subject-matter is arranged systematically to allow a comparison of parts which are homologous in different groups and also of parts which are functionally adapted to similar purposes.

Apart from occasional references to the results of electron microscopy this book deals only with general histology. It does not mention the results of specialized techniques that in recent years have come to be accepted as essential for the correlation of structure and function. Naturally, an author who attempts to cover such a wide field in a book of this size must be selective in his choice of material and the absence of reference to such specialized findings is forgivable on this ground. The selection of general material, however, is frequently uneven for no obvious reason. For example, in the chapter dealing with connective tissue, cells are dispensed with in four lines and although it is stated that these may consist of many types different in structure and function the only one named is the fibroblast. In contrast, in the same chapter, the matrix and fibres are dealt with in considerable detail and occupy approximately three pages. Again, in the section dealing with the reproductive system, no mention is made of the prostate or seminal vesicles. It is this unevenness which leads the reviewer to conclude that the student who will find the book most valuable is the one who has already acquired a basic knowledge of histology and comparative anatomy.

Approximately half of the illustrations are photomicrographs, the remainder being line drawings. In general most of these reach a satisfactory standard though some fall far short of what workers in human histology have come to expect. Such a shortcoming could be excused if the inferior illustrations were of biological material which was difficult to obtain. This, however, is not the case; for instance, the photomicrograph illustrating human blood is inferior because the blood film from which it was taken was obviously a badly prepared specimen.

The value of the book is increased by the inclusion of a short list of well-chosen references at the end of each chapter. These are by no means comprehensive but they should prove of value to the more serious minded student wishing to widen his knowledge of histology. In addition, a set of study questions has been added at the end of each chapter, the purpose of which 'is not only to test the student's knowledge but also to give him an opportunity to review and survey the contents of each chapter'.

This book is well written and it has to a large extent avoided the pitfall of being a compendium of facts related to different species. The author has undertaken a formidable task with considerable success. The shortcomings of the book are in part due to an attempt to contain the subject matter within reasonable confines.

F. R. JOHNSON

Anatomy of the Human Body. By R. D. LOCKHART, G. F. HAMILTON and F. W. FYFE. (Pp. ix + 697; 965 illustrations; 105*s.*) London: Faber and Faber. 1959.

The authors of this volume clearly think with Horace that 'whatever you teach be brief for minds grasp with readiness that which is said shortly and retain it firmly, all that is unnecessary overflows from the charged mind'. In the preface the authors state that the purpose of the book is to lighten the burden of the student of Anatomy. In pursuit of this aim they claim that the 'words are less than half and the illustrations more than twice the number in standard texts'. They, however, omit to say which standard texts.

They have, however, managed to produce something quite new in the matter of presentation. Whether this will lighten the burden of the student can only be judged by the experience gained from its use. A large number of the figures are in colour. In the section on the peripheral nerves pointers pass in profusion from the names in the text to the figures alongside. Whilst this may prove a success, it is also possible that it will make the reading irksome and irritating. In the section on the nervous system there are several coloured figures showing the nuclei and tracts in stereoscopic sequence in longitudinal extent. Some of these are excellent illustrations but there has been a tendency to introduce too much detail in many places. Many of the figures are too complex and certainly could not be reproduced by the student. The tendency has been to introduce too many features in the one figure. Whilst many of the illustrations are smaller than in many other standard texts they are drawn with clarity and are in general adequate. By the use of smaller illustrations it has been possible to place practically all of them on the same pages as the text which they serve. In the case of many of the radiographs, however, the size is quite inadequate; on one page alone there are as many as thirty miniatures, and details of bone structure and architecture, which, after all, are as important as the existence of centres of ossification and epiphyseal plates, are largely lost.

Living Anatomy is dealt with in the appropriate places throughout the volume; several of the illustrations are from the well-known book on this topic by the senior author. No attempt is made to cover either histology or embryology although paragraphs and illustrations are given over, here and there, to microscopical structure. There are frequent references to the clinical applications of the topics discussed. The bibliography consists of a list of about fifty books, some of which are recommended for the charm of their language, 'not exactly an engaging feature in some modern books'.

The production is excellent and the publishers are to be complimented on what must have been in places the very difficult task of marrying the text and the illustrations. As already stated, the presentation is novel and the information contained in the volume is of the high standard of accuracy that one would expect from the Department of Anatomy at Aberdeen. Whether, in fact, the method of presentation and the profusion of figures, in places of small dimensions and often bristling with pointers, will meet with the approval of the student or even lighten the burden will have to await trial.

D. V. DAVIES

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

NOVEMBER 1959

The Annual General Meeting of the Society for the Session 1959–60 was held on Friday, 27 November 1959, in the Department of Anatomy, Middlesex Hospital Medical School, London, W. 1.

The retiring President (Prof. F. Goldby), the newly elected President (Prof. J. D. Boyd) and Professors R. D. Lockhart and J. J. Pritchard occupied the Chair at the various sessions.

The following are the authors' abstracts of the papers presented.

Experimental study of head morphogenesis: removal of optic vesicles.

By P. H. S. Silver. *Department of Anatomy, Middlesex Hospital Medical School, London*

The present communication consists of a preliminary survey of the results of experiments carried out in the chick embryo between the seven and fourteen somite stages. Results have been examined after up to 15 days' total incubation. At least forty results are reported.

Removal of the optic vesicles results in great reduction of the orbit, with corresponding reduction in the interorbital septum, not only in the vertical direction but also in the horizontal. The latter causes foreshortening of the upper beak, whose tip never reaches the end of the mandible. Nevertheless, the chondrocranium does not lose its tropitrabic form. In certain instances the upper beak may be reduplicated.

The inhibitory action of antimycin A on the chick embryo.

By J. McKenzie and J. D. Ebert. *Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.*

Antimycin A, an antibiotic produced by a species of streptomyces, is a strong metabolic inhibitor and has been used to investigate the metabolism of developing tissues of the chick embryo cultured *in vitro*. Previous workers, using the explantation technique described by Spratt, showed that this substance, in very low concentrations, causes degeneration of all mesodermal derivatives, including heart, while the brain remains free of gross developmental anomalies. Antimycin A was therefore considered a specific inhibitor of developing heart and mesoderm.

These experiments were repeated and the results confirmed, but the use of the New explantation technique and the application of the inhibitor *in ovo* reveal no specific action on any tissue in the chick embryo between 24 and 48 hr incubation. The effect of antimycin A appears to depend, in large measure, on which developing tissue the substance contacts first during the experiment. Histological examination of specimens recovered shows necrosis of brain and somites. After exerting an initial necrotic or inhibitory action on a tissue the effect of the drug ceases and those parts of the embryo which have escaped damage differentiate in as normal a fashion as possible. The significance of these results is discussed.

The effect of foetal hypophysectomy on the thymus. By J. G. Bearn. *Middlesex Hospital, London*

The pituitary gland of the rabbit foetus was removed at the 20th or 21st day of pregnancy, using the method described by Jost of intra-uterine decapitation. Preliminary observations have been made on five headless foetuses which developed for 7 days. The observation, first made by Jost, that the adrenal gland is reduced in size compared with control foetuses has been confirmed. The thymus gland was found to be significantly enlarged in all the headless foetuses, compared with the controls. The significance of this finding is discussed in relation to the functional activity of the foetal adrenal gland.

The effect of an increased gravitational field on the growth of rats. By F. L. D. Steel. *The London Hospital Medical College*

A centrifuge has been constructed capable of producing a field of 2·8–3 g. directly through the floors of rat cages, and six litters of albino laboratory rats have been divided into approximately equal numbers; half of them being kept in the machine for periods of up to a year. The centrifuge is driven by a constant-speed a.c. motor (Matthews, personal communication, 1956) and free access to food and water is provided. Animals have been introduced into the centrifuge at 26–74 days of age and controls have always been chosen from the same litter.

It has been found that the adult weight reached by spun rats (either measured directly or calculated) is significantly decreased, but that during certain periods the slope of the logarithm of weight increase is steeper. In one litter (spun from day–26) the tangents of the slopes are 111·5 and 85 (values of $\bar{x}$, $n = 4$) for spun and unspun rats, respectively, during the period day–40 to day–68. This represents a difference between the means of four standard deviations. Final body weight has been correlated with the dimensions of a long bone.

A species difference in the response of the thyroid to the addition of calcium and iodine to a meat diet. By M. G. Scott and Patricia P. Scott. *Royal Veterinary College and Royal Free Hospital School of Medicine, London*

Using a high protein, high fat diet of raw heart, we observed hypertrophy, hyperaemia and hyperplasia of the thyroids of kittens receiving the diet for more than 8 weeks.

This condition could be partly corrected by adding either sufficient calcium salts (gluconate or carbonate) to give a calcium/phosphorus ratio of 1 to 1, or iodine at the rate of 100 μg./day. When calcium and iodine were given at the same time as the supplements the thyroids remained normal.

This result conflicted with the findings of other workers on the effects of calcium and iodine on the thyroids of rats. We repeated our experiment using young rats instead of kittens.

Hypertrophy and hyperplasia of the thyroids of rats fed on heart and calcium were more severe than in rats fed on heart alone. On heart supplemented with iodine (8 μg./day) the thyroids were normal, but on heart supplemented with calcium and iodine slight hypertrophy occurred.

Thus additional calcium had a goitrogenic effect in rats on a meat diet, even in the presence of adequate iodine, in marked contrast to the effect of supplementary calcium on the thyroid of cats.

The lymphatics of the breast. By R. T. Turner Warwick. *Middlesex Hospital, London*

The following conclusions are the result of injecting dyes and radioactive tracers into breast tissue, and observing their transport to the regional nodes by the natural flow of lymph during more than eighty mastectomy operations and subsequently in the specimens.

(1) There is nothing unusual about the lymphatics of the breast; the trunk lymphatics tend to accompany the blood supply.

(2) The so-called subareolar plexus has not been found to play an important part in the pathway of lymph drainage from the resting breast parenchyma.

(3) The minute lymphatics of the deep fascia play no part in the lymphatic pathway from the breast, nor in the early spread of carcinoma.

(4) The distribution of the axillary and internal mammary lymph nodes is described and the proportional distribution of the breast lymph under normal circumstances discussed.

(5) The internal mammary chain is an important pathway of lymph drainage from both the lateral and the medial halves of the breast.

(6) The para-xiphisternal lymph nodes do not normally receive breast lymph, neither is there any contralateral drainage to the opposite axilla or internal mammary chain unless the ipsilateral drainage is blocked.

(7) The posterior intercostal nodes inconstantly receive breast lymph.

Movements of the hyoid bone relative to the thyroid cartilage on neck flexion.

By G. M. Ardran and F. H. Kemp. *Nuffield Institute for Medical Research, University of Oxford*

It is recognized that the upper borders of the lamina of the thyroid cartilage pass beneath the hyoid bone with the elevation of the larynx during deglutition: we have found that the body and greater horns of the hyoid are aligned approximately in the transverse plane, with the superior borders of the laminae only just beneath the hyoid: there is no difference between the sexes. In the adult female the hyoid bone is inclined so that the greater horns are aligned approximately parallel to the lower border of the mandible and to the long axis of the laryngeal ventricles. The thyroid cartilage is situated about 1 cm. below the hyoid bone with the laryngeal prominence projecting just in front of the body of the hyoid bone. The thyroid cartilage in the male is usually relatively larger and its prominence projects relatively further forwards. When the female flexes the head and neck the hyoid bone is brought forward and is depressed over the thyroid cartilage until the lower border of the body is on a level with the laryngeal ventricles, the greater horns being at about 45° to the ventricles. In the male the hyoid bone is also brought forward, but the body is not depressed over the thyroid cartilage to the same extent. Certain males have female proportions in this region; but the relative range of movements between the hyoid and thyroid is not as great as in the female. The male speaking with falsetto voice brings the hyoid forward to the position seen in the female at rest. The position of the hyoid in relation to the thyroid in children of both sexes is similar to that in the female. The range of movement between the hyoid and the thyroid cartilage and the sex variations in their relative positions do not appear to have been previously described.

Electromyography of posterior vertebral muscles.

By J. Joseph and I. McColl. *Guy's Hospital Medical School, London*

The study of the posterior vertebral muscles by means of electromyography has usually been limited to the lumbar region. In the present investigation the right and left sides at eleven levels (alternate vertebral spines from S 2 to C 4) have been studied with surface electrodes and the subject in the standing-at-ease position. In twelve male subjects it was found that there was variable activity in the sacral region, little or no activity at the level L 5–L 3, marked or moderate activity at the level T 11–T 9, and usually little or no activity at the C 4 level. Sometimes there was more activity on one side than the other.

The main findings can probably be correlated with the relation of the line of weight to the transverse axes of rotation between vertebrae. Absence of activity is found if the line of weight falls behind these axes and moderate or marked activity if it falls in front.

Fate of the fracture 'Haematoma'. By M. Scott and J. J. Pritchard. *Queen's University, Belfast*

The fate of the extravasated blood has been studied in a series of closed fractures through the third metatarsal in the rat. Observations were made at intervals between 5 min. and 2 months after injury.

The blood clot infiltrating the periosteum and bone marrow adjacent to the fracture, and filling the fracture gap, appeared at first as a fine fibrin network, containing red cells indistinguishable from those within the blood vessels. In less than a week the red cells had broken down and most of the debris had been removed. In the periosteum and bone marrow the fibrin network was also quickly removed and replaced by connective tissue cells and blood vessels. The fibrin in the *fracture gap*, however, behaved very differently. It became converted into a coarse, stringy mass surrounded by fluid and then, adhering to the ends of the bones and the surface of the surrounding soft tissues, it formed the lining layer of a fluid-filled 'joint' cavity.

After 2 months callus had bridged the fracture in several places, but remains of the 'joint' space were still to be seen around the rough edges of the original shaft. By now, however, most of the fibrin had been removed and the 'joint' surfaces were cartilaginous.

It is believed that long persistence of the fibrin is both caused by, and is an adaptation to, mechanical instability of the fracture line.

Vascularization of long bones in oestrogen-treated mice. By M. Brookes and E. G. Lloyd. *University of Liverpool*

Immature male and female mice, weaned at 21 days, were subjected to daily subcutaneous injections of 40 i.u./g. body wt. oestradiol monobenzoate. The mice were sacrificed at intervals of 2, 4, 6 and 8 weeks and injected intravascularly with india ink, Micropaque and Thorotrast. Specimens were examined radiologically and histologically. Radiological study of the vascularization of mouse long bones was facilitated by microradiography of 250μ sections of celloidin-embedded material.

The results indicate that the formation of endosteal new bone in oestrogen-treated mice does not completely disrupt the vascular pattern and organization of the bone marrow. Although the vascular areas of the medulla are encroached upon, the vascular scaffolding persists even in advanced states of marrow ossification.

The olecranon epiphyses. By C. J. Porteous. *St Bartholomew's Hospital Medical College, London*

Theories concerning the evolution of certain long-bone epiphyses have recently (1958) been reviewed by Barnett & Lewis. Any evaluation of the morphology of the human olecranon demands a preliminary and accurate recognition of its normal epiphyseal constitution. Regarding this, current accounts are conflicting, and even mutually exclusive. Osteological and radiological inquiry establishes the constant presence of two discrete olecranon epiphyses, one articular in nature, the other of the traction variety. Evidence indicative of the normal pattern of olecranon ossification in man and in certain other primates is submitted and the phenomenon of so-called 'patella cubiti' is interpreted in the light of the anatomical findings.

Glycogen fractions and their role in the histochemical detection of glycogen. By W. J. C. Wilkinson and J. H. Kugler. *The University of Sheffield*

The demonstration of tissue glycogen by the iodine method has been known for at least 104 years. Since then many methods have been described but there is no real consensus of opinion regarding the best technique (including fixation) for the histochemical detection of glycogen. By using a technique that involved following glycogenolysis in guinea-pig liver it was possible to take liver specimens for histochemical and biochemical assay simultaneously. The total glycogen content of the specimen was estimated by the anthrone method and a determination of the acid-soluble fraction, using trichloroacetic acid as solvent, was also made. For the histochemical detection of glycogen the tissue was stained by Gomori's hexamine silver technique, the periodic acid Schiff method or with Best's carmine. Precise correlation between the total glycogen content, the acid-soluble glycogen fraction and the histochemically demonstrable glycogen in the liver specimens could then be made. On comparing the histochemical picture with the known amounts of both the total and the acid-soluble glycogen fraction, it was found that it is only the acid soluble fraction of the tissue glycogen that is demonstrated by these histochemical techniques.

The experimental demonstration of zonulysis by Alphachymotrypsin *in vitro*.

By S. J. CREWS. *London Hospital and Moorfields Eye Hospital*

Alphachymotrypsin is a proteolytic enzyme of considerable value in ophthalmology since some 18 months ago Dr J. Barraquer of Barcelona reported that it weakens the zonule or suspensory ligament of the lens, thus facilitating removal of the lens with its capsule intact (intracapsular extraction). Though now in world-wide use clinically, the experimental work published to date does not appear to be very extensive. Initially, the enzyme was thought to have an almost specific action on the zonule but this probably depends on many technical factors.

Complete lysis of the zonular fibres was presumed to occur but evidence is accumulating that in the usual dilutions most of the fibres remain intact but rupture easily, possibly due to an action on the controversial interfibrillary cement substance. Further basic studies are essential to elucidate this problem, and this paper is concerned with the various microdissection techniques developed in order to prepare a specimen of zonule with the minimum of trauma, which could then be carefully examined under controlled conditions suitable for enzyme work. Preliminary results show absence of complete zonulysis; a milk-clotting test can quickly confirm that the enzyme remains active.

A preliminary report of a difference between juvenile and adult erythrocytes.

By W. K. METCALF and K. W. KEOHANE. *The University, Bristol*

During the course of an investigation into the replacement of foetal by adult haemoglobin, it was found that the haemoglobin of cord blood was more susceptible to methaemoglobinization by sodium nitrite solutions than is adult haemoglobin treated in the same way. Further investigation showed that there is a gradual change in the sensitivity of the erythrocytes during childhood and a very rapid increase in their resistance at about the time of puberty. This change seems to be due to the gradual replacement of sensitive cells by cells of increased resistance.

To date we have very little evidence of the cause of the differing sensitivities of juvenile and adult cells to nitrite, but there would appear to be four possibilities. The difference may be in the plasma, in the cell membrane, in the haemoglobin itself or in some other intracellular constituent. These possibilities are discussed.

Observations on extracted and intracellular ferritin. By A. R. MUIR.

University of Edinburgh

The protein ferritin with a M.W. of 465,000 and containing 23 % of iron, can be extracted from iron-storage tissues. Examination of this pure protein in the electron microscope shows that it consists of an almost spherical molecule, 100 Å. in diameter, of apoferritin with a central iron-containing 'nucleus' 50 Å. in diameter. The central mass of inorganic iron consists of six spherical micelles arranged at the corners of a regular octahedron. The random orientation of this structure produces a characteristic variety of images.

In ultra-thin sections of spleen, liver, duodenum and bone marrow from numerous mammals and spleen from the toad, dense particles 50 Å. in diameter can be recognized. At higher magnifications, these particles show the same variety of images as isolated ferritin. In cells the ferritin is mainly distributed throughout the cytoplasmic ground substance and, when present in large amounts, aggregates up to 2 μ in diameter are formed. A few ferritin molecules are present in the nucleoplasm and in the matrix of mitochondria.

Observations on transneuronal degeneration in the monkey lateral geniculate nucleus. By MARGARET R. MATTHEWS, W. M. COWAN and T. P. S. POWELL. *University of Oxford*

The process of transneuronal cell degeneration has been studied in the lateral geniculate nucleus in twelve macaques at intervals varying from 4 days to 4 months after unilateral eye enucleation. Measurement has shown that as the neurons in the affected laminae become smaller, the cytoplasm shrinks relatively more than the nucleus or nucleolus. This shrinkage occurs in two phases, separated by an interval of relative stability. An early rapid phase, which is complete within the first week, affects the laminae receiving crossed optic fibres (1, 4 and 6) earlier (within 4 days) but less severely than those receiving uncrossed fibres (2, 3 and 5). In the later phase, which begins towards the end of the first, or during the second, month there is no consistent difference in behaviour between the crossed and uncrossed laminae over the period studied. By 4 months the mean area of the atrophied neurons has fallen to 60 % of normal. No indication of loss of neurons has been found until 4 months after eye removal, when there appears to be a slight but significant cell loss in lamina 6.

Cortico-striate projections in the rat. By K. E. WEBSTER. *University College, London*

Cortical lesions were made by suction or undercutting in sixteen adult albino rats. The resulting degeneration was stained according to the methods of Nauta (1952) and Nauta Gygax (1954).

In three other animals cortical lesions were made and after 10 days' survival the brains were prepared for staining by the Swank–Davenport–Marchi technique.

The Nauta methods showed degenerating fibres leaving the internal capsule and entering the striatum. These degenerating elements were orientated approximately perpendicular to the axons of the internal capsule, suggesting that they are collaterals. The extra-capsular course is complex, and nests of degeneration were observed in the striatum. These findings and the negative result of the Marchi material are strongly suggestive that these fibres are not axons of passage.

The projections originate from widespread areas of the cortex, including the occipital regions. The present evidence indicates that the connexion is topographically organized, both antero-posteriorly and latero-medially. The pattern is distorted, especially in the wedge-shaped posterior regions of the striatum.

Golgi-rapid preparations have shown collaterals leaving internal capsule axons and entering the striatum. It has not been possible to locate the cell bodies of the parent axons (cf. Cajal, 1893).

The structure of myelin sheaths in the central nervous system. By A. PETERS. *University of Edinburgh*

An electron microscope study has been made of myelinated fibres in the optic nerve and spinal cord of tadpoles (*Xenopus laevis*). The arrangement of the myelin in the central nervous system is essentially similar to that in peripheral nerves; the lamellae are arranged in a spiral which begins at an internal mesaxon. The single difference occurs on the outside of the sheath. No external mesaxon is present, the spiral terminating by the separation of the two apposed membrane surfaces, which form the lamellae, to enclose a tongue of cytoplasm from the myelin-forming glial cell. This tongue is the only cytoplasm present on the outside of the sheath. Because of the absence of cytoplasm over most of their surface, frequent direct contacts are made between adjacent myelin sheaths. Such contacts between the external surfaces of the membranes result in the formation of an interperiod line between the apposed sheaths, similar to that produced when the surfaces of the membranes giving rise to the internal mesaxon come together. The early stages in the formation of the sheath are similar to those in peripheral nerves.

The distribution of cholinesterase-positive nerve fibres in the human uterus. By R. E. Coupland. *Department of Anatomy, Queen's College, Dundee, University of St Andrews*

Human pre-ganglionic sympathetic and pre- and post-ganglionic para-sympathetic nerve fibres can be demonstrated readily in tissue sections by a histochemical method used for the localization of cholinesterase. Using a modification of the Koelle and Friedenwald technique, the distribution of cholinesterase-positive nerve fibres in the uterus and Fallopian tubes has been observed. The material used consisted of thirty hysterectomy specimens obtained from subjects aged 24–76 years. The findings indicate a regional distribution of cholinesterase-positive fibres in the uterus.

In the body of the uterus fibres are few and are confined to the myometrium, where they are occasionally associated with muscle fibres, or with the adventitia of blood vessels. In the cervic cholinesterase-positive nerve fibres are abundant and are observed in both the fibromuscular coat and the endometrium. Cervical blood vessels, unlike those of the body of the uterus, are richly innervated by cholinesterase-positive fibres. A plexus of strongly positive nerve fibres and multipolar cells lies in the deeper part of the endocervical mucous membrane and the adjacent part of the fibromuscular coat of the cervix.

The change in the pattern of distribution of cholinesterase-positive nerve fibres in the uterus is relatively abrupt and occurs at the level of the internal os.

In the Fallopian tubes moderate numbers of cholinesterase-positive nerve fibres are observed in both the myometrium and in the mucous membrane.

The findings are discussed.

Neurohistological and histochemical observations on nerve-endings in the nose of the hedgehog and mole. By P. Alberti and N. Cauna. *Department of Anatomy, King's College, Newcastle-upon-Tyne*

Nerve-endings in the hairless and hairy skin of the nose were stained by a modified Bielschowsky–Gross silver-impregnation method; cholinesterase activity was demonstrated by a modified Koelle's histochemical technique. For histochemistry, tissues were fixed in neutral 10% formalin solution from 3 to 4 hr. at 4° C., frozen sections were cut and incubated from 1 to 18 hr. at pH 5·2 to 5·4. Eserine controls were used with both substrates.

It was found that intra-epidermal nerve fibres and the related epidermal cells gave negative reactions with acetyl- and butyryl-substrates. Papillary endings and the nerve fibres of the preterminal plexus showed a positive reaction with acetyl-substrate only. A perivascular plexus of corial blood vessels was also revealed by the acetyl-substrate. Capillaries showed negative reaction.

In the hairy skin positive acetyl-substrate reaction was observed within the regions of the nerve plexuses surrounding the hairs. The blood vessels of the sinus hairs were negative. Musculi arrectores pilorum and striped cutaneous muscle fibres showed a positive reaction with acetyl-substrate in the region of neuro-muscular junctions.

Contrary to the nerve-endings of the human digital skin, those in the nose of the mole and hedgehog showed no pseudocholinesterase under similar experimental conditions.

Nissl substance and cholinesterase in developing neurones. By A. F. Hughes and P. R. Lewis. *Anatomy School, Cambridge*

The ventral horn of the anuran tadpole is clearly distinct from the mantle layer of neuroblasts from which its cells are derived. Their differentiation can readily be followed stage-by-stage. We have traced the distribution of ribonucleotides by means of the ultra-violet microscope and the appearance of cholinesterase by a modified Koelle technique. At first, the RNA is uniformly dense, but shortly before the enzyme can be detected within the cytoplasm, the ultra-violet-absorbing material becomes arranged in a series of hollow vesicles, the centres of which are occupied by non-absorbing substance. As the developing neurone enlarges by the accumulation of more and more of the latter, the RNA assumes the pattern typical of Nissl substance. If the limb buds are removed and the axons of the ventral horn cells thus severed, the non-absorbing substance disappears once again from the cytoplasm, and the whole cell shrinks markedly.

Acid triphenylmethane dyes in neurological staining. By E. Gurr and M. A. MacConaill. *University College, Cork*

The present study investigates the affinities of some half dozen dyes belonging to the same chemical group as acid fuchsin. It appears that the uptake of a given dye by various tissue elements in cat spinal cord depends in great part upon the molecular weight of the dye. Chemical factors play a special part in combined staining with pairs of these dyes. The results are in accord with previous work by M. A. MacC. and with the most recent histochemical studies of the axonic proteolipid cuticle carried out by quite different methods; and with earlier studies by E. G. on the influence of molecular weight on staining.

The investigation showed that a good and simple differentiation between medullated fibres and other neurological elements is given by the combined use of acid fuchsin and light green. It was found that these two dyes combine to form three different compounds which were isolated in solid form by E. G. One of these, a blue dye, gives a very good two-colour differentiation when used in simple aqueous solution, medullated axons being coloured red, other material light blue, nuclei dark blue. A clear picture (red) is given by RBC and neuronic nucleoli, both in freshly preserved and dissecting-room material.

Placodal contributions to the glossopharyngeal nerve and the developing carotid body. By E. H. Batten. *University of Bristol*

In sheep embryos with thirty to thirty-eight somites the tip of the IX neural crest receives small cellular spurs from the epibranchial placode. The petrosal ganglion then appears in the central part of the crest and its distal part, representing the rudiment of the carotid nerve, becomes intimately associated with the placode, so that migrating cells are no longer clearly seen. Nevertheless, the sustained mitotic activity in the placode, the local loss of its basement membrane, the impression of a cellular emigration and the increasing cellularity of the apposed tip of the carotid nerve suggest that the latter continues to receive placodal cells during the 8–11 mm. stages. By this time the placode becomes buried in the cervical vesicle 2 and the nerve soon detaches from it. Finally, in the 11·5–16 mm. stage, spurs of migrating placodal cells are again conspicuous as they traverse the mesenchymal gap on their way to the carotid nerve. A redistribution of placodal cells within the carotid nerve in 13 mm. embryos produces transient cellular accumulations which can be traced into the mesenchymal primordium of the carotid body. About the 15 mm. stage emigrating preneuroblasts from the petrosal ganglion apparently follow the same route into the carotid body.

Further observations on the closure of the secondary palate in two strains of rat. The effect of administering vitamin A alone or in combination with cortisone on the 10th and 15th day of gestation. By J. W. S. Harris. *The London Hospital Medical College*

The administration of large doses of vitamin A to pregnant rats between the 7th and 13th days of gestation has been reported to result in a varying incidence of cleft-palate in the young. In most series treatment was given over several consecutive days and the young were examined in late pregnancy. The simultaneous injection of cortisone has been reported to potentiate the effect of the vitamin.

In the present work single doses of 150,000 i.u. vitamin A were administered either alone, or in combination with 25 mg. cortisone to forty-eight pregnant rats on the 10th and 15th days of gestation. In both strains (Chester Beatty and Lister Institute) the palate normally closes during the 16th day (day of sperm recovery = day 0). Embryos recovered on the 17th and 21st days were weighed and the heads dissected or serially sectioned.

In both strains, treatment on the 10th day disturbed the development of cartilage. Affected embryos were characterized by a varying degree of microstomia, pointed snout, prominent lid-less eyes, ankylosis of the jaws and rudimentary palatal processes. There was a high incidence of complete cleft-palate with both forms of treatment.

Treatment on the 15th day resulted in a smaller incidence of complete cleft-palate, which was increased by the addition of cortisone. The palatal processes were well developed and there were no gross abnormalities of cartilage. In embryos recovered on the 21st day there was a significant increase in the number of closed palates, suggesting that treatment on the 15th day delayed palatal closure beyond the normal time.

Mating and birth of a camel (*Camelus dromedarius* L.). By W. R. M. Morton. *Queen's University, Belfast*

Travellers' tales suggest that camels in a pack-train will mate while standing buttock to buttock, but Leonard (1894, *The Camel*) denies the accuracy of these reports. The present observations on the untrammeled dromedary showed that coitus occurred with the female in the 'kneeling' position. The male mounted the female with his forelegs astride her back, and hindlegs in a squatting position immediately outside hers. Coitus lasted from 7 to 10 min. and was repeated once or twice after intervals of 20–30 min. if the animals were left in the paddock. Gestation, in this instance lasted 417 days from the date of the last coitus; the first stage of labour lasted 10 days, possibly due to the presence of observers, but the second and third stages together only lasted 2¾ hr. The chorion is covered by small tufts which are not uniform in size all over this membrane. Microscopically these tufts indicate that the maternal–foetal relationship of the placenta is an epithelio-chorial one. A short film of coitus and labour was shown, followed by a short description of the foetal membranes.

The antimesometrial decidua and decidua capsularis of the rat placenta. By D. Bulmer and A. D. Dickson. *University of Aberdeen*

At the 9-day stage the antimesometrial decidua is formed mainly of large cells with a high content of cytoplasmic nucleic acid. It is surrounded by a basophilic 'capsular' layer, which separates it from the uterine lumen or from the stroma of the uterine wall in the regions which are not penetrated by the lumen. The capsule, which has not been reported previously in the form we describe, has numerous small nuclei embedded in it. At the 10-day stage its staining reactions, apart from a marked basophilia, resemble those of fibrinoid. Apparently similar material accumulates in the cytoplasm of many of the anti-mesometrial decidual cells. Except near the antisometrial pole, the capsule persists at the 12-day stage, surrounding a loose degenerate layer which contains numerous nuclear remnants. Inside this, the foetal giant cells begin to show signs of degeneration. The degenerate layer is rich in ribonucleic acid and the capsule retains its basophilia. By the 15-day stage only a very thin layer, with few nuclear remnants, separates the uterine epithelium from Reichert's membrane.

Cell movements through the capsule of the chorio-allantoic placenta in the rat.

By A. D. Dickson and D. Bulmer. *University of Aberdeen*

The capsule separating the mesometrial decidua from the uterine tissues at the base of the mesometrium has a central perforation, through which the supplying arteries pass inwards to the chorio-allantoic placenta. There appear to pass outwards through this perforation, in succession, metrial gland cells, endovascular plasmodium (within the arteries), junctional zone trophoblast and foetal glycogen cells. The apparent outward passage of metrial gland cells and their association with trophoblast pose the question of the composition of the metrial gland, which is generally believed to be entirely of maternal origin. The exit of the presumed and known foetal tissues is associated with the shortening of the mesometrial-antimesometrial axis of the chorio-allantoic placenta incurred in the disappearance of the decidua basalis. It results in a mixing of foetal and maternal tissues at the base of the mesometrium in the later stages of gestation.

The development of the anterior cerebral artery in the rat.

By D. B. Moffat. *University College, Cardiff*

The development of the anterior cerebral artery has been studied in 150 injected rat embryos, ranging in size from C-R.L. 2·5 mm. up to full-term foetuses. In the youngest embryos, the principal artery supplying the cranial extremity of the brain is the primitive maxillary artery which passes ventral to the optic outgrowth. This vessel breaks up to form a bilateral plexus which is joined by the primitive olfactory artery. The primitive maxillary artery persists until the 6–8 mm. stage and then diminishes in size, while at the same time a new recurrent branch of the primitive olfactory artery differentiates out of the plexus, which has approached the mid-line. This branch runs ventrally and caudally, cranial to the optic stalk, and, together with the primitive maxillary artery, helps to form a complete arterial ring around the base of the stalk. From the cranial aspect of the recurrent branch, a plexus of vessels converges towards the longitudinal fissure, and from this plexus one or more channels become apparent on each side to form the main part of the anterior cerebral artery, the stem of origin of which is formed by the proximal part of the recurrent branch. The rest of the recurrent branch, and the primitive maxillary artery persist as small branches of the circle of Willis.

The lung of *G. domesticus*: secondary bronchi.

By D. C. Payne and A. S. King. *University of Bristol*

The main branches of the primary bronchus, i.e. the secondary bronchi, have long been known. Yet there is still disagreement in the literature even about major details like their number and orientation. Investigation of numerous lungs of *G. domesticus* has given the following details for this species.

(1) The 'ventromedial' secondary bronchi, 'ventrobronchi', or 'entobronchi': four large tubes arising from the dorsomedial wall of the cranial third of the primary bronchus.

(2) The 'dorsomedials', 'dorsobronchi', or 'ectobronchi': next in size; seven to nine, usually eight, tubes arising in a line along the dorsal wall of the caudal two-thirds of the primary bronchus; calibre reduced caudally.

(3) The 'laterals, or laterobronchi': six to eight, usually eight, smaller tubes arising opposite the 'dorsomedials' in a line along the ventral wall of the primary bronchus; calibre progressively reduced, the seventh and eighth being only the size of tertiary bronchi.

(4) The 'dorsals': about twenty-five to thirty small tubes about the size of tertiary bronchi, arising along the lateral wall of the caudal two-thirds of the primary bronchus.

In this species several of the names which are commonly used for the four groups of secondary bronchi are misleading since they suggest an incorrect orientation.

Further studies on the endocrine pancreas of the metahypophyseal diabetic cat.

By J. D. LEVER and MARJORIE JEACOCK. *Departments of Anatomy and Biochemistry, University of Cambridge*

The daily administration of growth hormone to cats produces the following changes in the β-cells of the islets of Langerhans. Within 10 days there is a marked depletion of the characteristic β-cell granules (judging from electron micrographs) and the simultaneous appearance of traces of glycogen. Both these changes are progressive with time up to the establishment of unequivocal metahypophyseal diabetes. In this state the β-cells contain no characteristic granules and because of the large quantities of intracellular glycogen, nuclei tend to be eccentric and other organelles are displaced peripherally and into a perinuclear position.

The clinical response to tolbutamide is not always satisfactory but when this is positive it is reflected in a reversal of the cytological changes described above and the restoration of normal β-cells.

The role in the secretory process of certain organelles can be discussed in the light of these observations.

Incorporation of tritiated thymidine by epithelial cells of duodenum and seminal vesicle.

By A. W. ROGERS. *University of Birmingham*

Firket (1958), studying fibroblasts in tissue culture, has shown that thymidine is taken up by cells in late interphase, at a time when their DNA is doubling in amount. 6–11 hr. after thymidine uptake ends in a cell, it enters mitotic prophase. Several workers claim that thymidine is not utilized at any other time in the cell cycle. Pelc (1959) has challenged this latter conclusion, by showing the seminal vesicle epithelium takes up tritiated thymidine in greater quantity than would be expected from its mitotic rate. He postulates a metabolic turnover of DNA independent of mitotic activity.

In the present study, nine male mice were injected with 20 μc. each of tritiated thymidine, and sacrificed at half-hourly intervals. Autoradiographs of duodenum show a very short period between thymidine uptake and mitosis *in vivo*: many labelled mitotic figures were seen $1\frac{1}{2}$ hr. after injection. The increase with time in the number of labelled epithelial cells in duodenum and seminal vesicle was presented, and Pelc's hypothesis was discussed in the light of these results.

The structure of epidermal keratin.

By R. I. C. SPEARMAN. *Dermatology Department, University College Hospital Medical School, London*

Mammalian stratum corneum keratin is more flexible than reptile scale keratin. Keratinized cells of the tail scales of rodents resemble lizard and snake epidermal scales in many ways. Differences between the flexible and rigid types of keratin can be shown by fluorescence microscopy and histochemical techniques.

Epidermal scale keratin retains much of the original cell contents, but has no haematoxylin-stainable nuclei. Parakeratotic keratin resembles scale keratin but altered cell nuclei are retained. Scale keratin is usually not formed from a granular layer. Unlike scale keratin, flexible keratinized cells have lost their cell contents, and are little more than keratinized cell membranes. Flexible keratin is formed from a granular layer, and appears to be associated with hair follicles in mammals.

The keratinized cell membranes can be demonstrated by treating sections with 3% peracetic acid and afterwards staining with methylene blue, or the fluorochrome thioflavine T. The intensity of staining seems to be related to the amount of cystine (S–S) present. The hollow structure of flexible keratinized cells enables them to be deformed without loss of strength. Scale cells and hair cortical cells are solid structures and form a more rigid keratin.

Absence of muscle spindles in muscles of rats reinnervated during development.

By J. Zelená and P. Hník. *Institute of Physiology, Czechoslovak Academy of Sciences, Prague*

In previous experiments rapid disintegration of immature spindles was found in denervated muscles of new-born rats (Zelená, 1957). During reinnervation spindles in these muscles must therefore be formed *de novo*, if at all.

To find out whether spindles can regenerate under these conditions, reinnervation was studied in extensor digitorum longus of rats in which the sciatic nerve had been crushed unilaterally immediately after birth.

After 5 months only occasional atypical spindles were found in the reinnervated muscles, average number of spindles being 0·5 in one muscle. On the other hand, the control muscles contained 32·3 ± 2·32 muscle spindles. During normal development the differentiation of spindles was dependent on afferent innervation; the number of sensory fibres was therefore counted in regenerated nerves to determine whether the absence of spindles was due to an insufficient regeneration of afferent fibres. After bilateral anterior rhizotomy of segments L_2–S_1 and degeneration of efferent nerve fibres the number of reinnervated afferent fibres in the deep peroneal nerve was 74·1 ± 13·8% of the control side. These sensory fibres reached the muscles and branched there, but only occasional atypical spindles were found in reinnervated muscles, while the average number of spindles in control muscles was 29·25 ± 1·11 in anterior tibial muscles and 32·75 ± 1·87 in extensor digitorum longus.

It can be concluded that, in spite of reinnervation by sensory fibres, spindles do not regenerate in muscle reinnervated at a late stage of development.

The circulation in mammalian skeletal muscle.

By T. E. Barlow, D. N. Walder and A. L. Haigh. *Departments of Anatomy and Surgery, Medical School, King's College, Newcastle-upon-Tyne*

Evidence has been previously presented (Barlow, Walder & Haigh, *J. Anat., Lond.*, **92**, 1958) indicating that arteriovenous anastomoses are not to be found in the voluntary muscle of the cat or rabbit, nor have the through ways or preferential channels described by Zweifach & Metz (*Angiology*, **6**, 1955) been observed in these animals. Another explanation is therefore required to account for the fact that during the intravenous infusion of adrenalin into man (Walder, *Clin. Sci.* **12**, 1953) and cat (Miller & Wilson, *J. Physiol.* **43**, 1951) the expected linear relationship between (*a*) the rate of clearance of ^{24}Na previously injected into a muscle and (*b*) the rate of blood flow through that muscle, is not found.

If a dose of ^{24}Na is injected into the nutrient artery of the semi-isolated biceps of the cat and the clearance curve is then plotted, two components to the curve can be detected, one representing a slow clearance of ^{24}Na and the other representing a fast clearance. This suggests that there may be two regions within the muscle, one having a good blood supply and the other having a poor supply. Auto-radiographic studies of muscles made at different times during these experiments show that at first ^{24}Na is diffusely spread throughout the muscle, but later remains principally in areas where tendon and aponeurosis are situated, thus indicating that tendon and aponeurosis may have a poorer blood supply than muscle fibres. Arterial injections of indian ink into the biceps confirm this.

By using very small volumes (0·05 ml.) of ^{24}Na to make localized injections into regions where either muscle fibres or tendinous fibres predominate it has been possible to show that subsequent intravenous infusions of adrenalin increase the rate of ^{24}Na clearance from muscle fibres but decrease the clearance rate from tendinous fibres during the increase in total muscle blood flow.

It is therefore suggested that in skeletal muscle there are two circulations, one serving muscle tissue and the other confined to intermuscular septa and tendons, and that previous discrepancies between blood flow and ^{24}Na clearance were due to lack of realization of this fact.

DEVELOPMENT OF THE HUMAN PLACENTA IN THE FIRST THREE MONTHS OF GESTATION

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AND

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INTRODUCTION

In spite of an extensive literature, going back to the eighteenth century, our knowledge of the details of the structure of the human placenta is still most fragmentary. This is due partly to the fact that much of the work published on its histology has been concerned with late stages of its development and predominantly with placentae separated from the uterus. So far as the earlier stages are concerned, individual investigators have had access only to a very limited number of specimens. Thanks to the invaluable collaboration of a number of obstetricians and pathologists over a number of years, the authors now have in their possession an extensive collection of pregnant uteri at all stages of gestation from about the 11th day after fertilization to full term. Serial sections of a part or the whole of the implantation sites and placental areas of these specimens have been prepared. Consequently the authors have been able to make a full survey of the structure of the human placenta throughout the period of gestation. The present contribution deals with the establishment of the placenta and with its development up to the 60 mm. c.r. length foetal stage. By the end of this period, the general structure of the placenta is essentially that which it will possess throughout its later development. Our aim is to provide a general survey of the early history of the human placenta as a background to more detailed consideration, in later communications, of particular aspects of the relationship between mother and foetus.

In a classical review, published in 1927, Grosser summarized the then existing knowledge of implantation and development of the placenta in Man. The early stages of human development available for study at that time were unsatisfactory. Many of the specimens were incomplete or even fragmentary. For example, only five sections of the Miller ovum (Streeter, 1926) were available for study, and some classical descriptions were undoubtedly based on pathological material (e.g. T. B. 1, Bryce & Teacher, 1908). Since Grosser's review there have been outstanding contributions on the early stages of human implantation and placental development. Especially noteworthy are the investigations of Hertig and Rock since 1941 (summarized by Hertig, Rock & Adams, 1956). To a lesser extent, a number of other investigators have also added descriptions of specimens at these stages (see Hamilton & Boyd, 1950; Hamilton, Boyd & Mossman, 1952; and Mazanec, 1959, for summaries). Additions in the last thirty years to knowledge of stages of human placental development later than the 18th post-ovulational day and especially of the fully established *in situ* placenta have been much less extensive. Indeed, as indicated in earlier communications (Boyd & Hamilton, 1950; Hamilton & Boyd, 1951), there is still disagreement and doubt on a number of fundamental points relating to the structure and function of the human placenta at all stages of its development.

The numerous contributions from the Carnegie Laboratory of Embryology on implantation of the blastocyst and on placental development in the macaque monkey (Wislocki & Streeter, 1938; Heuser & Streeter, 1941; Ramsey, 1949, 1954*b*) have provided a remarkably clear picture of foetal-uterine relationships in this primate. In spite of numerous differences, in detail, including a fundamental one in implantation itself, from the conditions obtaining in Man, the data on macaque development constitute an essential comparative basis for any serious consideration of the conditions in the human uterus during gestation. Throughout the study of our material we have found it necessary at every stage to make comparison with the equivalent one in the macaque. It must be stressed, however, that, as Ramsey (1954*a*) has stated with regard to placental circulation in the two species, the findings in the macaque are not replicas of those found in Man. Conclusions on the morphology of the placenta in the one species cannot, therefore, be transferred automatically to serve as the basis for interpretations of the conditions in the other primate.

A special problem in the description of the development of the foetal components of the placenta is posed by the various appearances presented by the trophoblast at different stages of development and in different regions of the blastocyst wall. Part of the difficulty resides in the variety of terminologies used by investigators in their descriptions. Recently, the authors (Boyd & Hamilton, 1960) have discussed this terminological problem, and it is not considered necessary to review the matter in detail in the present contribution. As the suggestions on terminology put forward by us, however, will be used in our subsequent descriptions, a brief summary of them is required. It must at once be stressed that this terminology is essentially a topographical and descriptive one. Briefly, those cells constituting the wall of the unimplanted blastocyst, and which are individual cellular units, together constitute the *blastocystic trophoblast*. All of the trophoblast of later stages is derived from it. During the early stages of implantation the blastocystic trophoblast differentiates into *primitive syncytiotrophoblast* and *primitive cytotrophoblast*. The former, receiving increments over a considerable period from the latter, is, by the development of the cytotrophoblastic, or trophoblastic, shell, separated into two varieties. First, there is the *definitive syncytium* which forms a covering for the villi and lines the lacunar and, later, the intervillous spaces. Secondly, there is syncytium on the maternal side of the shell which we call *peripheral syncytium*. Both varieties of syncytium are augmented until late stages of placental development by further contributions from the related cytotrophoblast. This last named tissue gives origin to: (1) the *villous cytotrophoblast* (Langhan's layer); (2) proliferations at the tips of the villi which constitute the *cytotrophoblastic columns;* and (3) the *trophoblastic shell* itself. It must be stressed that although there is good evidence that cytotrophoblast from any of the three named subdivisions can give origin to syncytium, reverse differentiation of the latter into the former seems, in normal development, not to occur.

Until the contribution of Spanner (1935) it was generally accepted that, for the most part, the decidual arteries open into the intervillous space, at or near the margins of the septa which separate the cotyledons, and that the uterine veins communicate with the intervillous space through orifices in the interseptal zones of the basal plate. Indeed, Bumm (1893) considered that the openings of the decidual arteries are usually situated quite far down the septa and well away from the basal plate itself. Spanner, however, as a result of observations on placentae injected *in situ*, concluded that the arteries open into the intervillous space solely through the basal plate and that the blood is only drained away from this space through a specialized peripheral region of it called the marginal sinus. Stieve (1942) and we, ourselves (Hamilton & Boyd, 1950; Boyd, 1956), have criticized Spanner's concept of the intervillous circulation and have denied the existence of a constant marginal sinus in Spanner's sense of the term. In particular, it can be shown that there are frequent venous openings through the whole of the basal plate. Ramsey (1954*b*, 1956*a*, *b*) has also shown that uterine venous connexions with the intervillous space can be found in all regions of the uterine surface of the macaque placenta. She points out, indeed, that, in this monkey, such connexions are even less prominent at the margin of the placenta than in Man; she attributes this difference to the superficial implantation in the macaque which 'precludes contact between the placental margin and the endometrium when vascular connexions are being established'.

In view of the physiological and clinical importance of the circulation in the intervillous space, and of the conflict of opinion on it, special attention has been given to the morphology of the circulatory arrangements in the placenta at all stages of our study.

MATERIAL AND METHODS

The available *in situ* specimens in the age group with which we are here concerned have, for the most part, been obtained from hysterectomies (Table 1). In most instances the co-operation of the obstetrician was such that the intact uteri were available within a short time after their removal. Fixation, therefore, has frequently been under optimal conditions. One specimen (42 mm. stage) came from a woman who was accidentally electrocuted. Several others were obtained from material removed during diagnostic curettage. Further details on the history of the younger specimens are given subsequently. In these earlier stages, the embryonic age has been estimated from the coital and menstrual history and from the general state of development of the trophoblast and embryo. In the older specimens, the crown-rump measurement has been used in the assessment of the age but, when fully available, the reproductive history has also been taken into account.

In addition to the *in situ* material we have an extensive collection of sections through early placentae obtained from hysterotomies, curettages and abortions. For certain histological and cytological purposes this disparate material has proved very useful as a supplement to our *in situ* specimens. We also have available a number of non-pregnant uteri from hysterectomies often at known stages of the uterine cycle.

The earliest developmental stage at our disposal is the Barnes embryo, of which a brief description has already been published (Hamilton, Barnes & Dodds, 1943). This specimen, almost completely implanted in the endometrium, was found in a uterus obtained by hysterectomy on the 25th day of a menstrual cycle. Previous cycles were regularly 28 days in duration and the menstrual periods lasted 2–3 days. A history which appears to be reliable establishes that coitus had taken place on the 10th and on the 12th days previous to the operation. Consequently, the maximum conceptional age is not more than 12 days. The uterus was opened at the attachments of the broad ligaments. On examination with a low-power binocular microscope an elevation (Pl. 2, fig. 2) was seen on the posterior uterine wall as a slightly raised translucent area, clearly demarcated from the surrounding endometrium. After fixation, with Bouin–Allen fluid, the area became opaque but was still elevated above the level of the surrounding endometrium. A block of uterine tissue containing the swelling was removed and, after double embedding, was cut serially at 7μ. After sectioning, the dimensions of the chorionic sac are approximately 0·931 × 0·770 × 0·737 mm.

Table 1

Specimen	Age	History
Barnes	11–12 days	Hysterectomy
Missen	14 days	Curettage
Gar	18 days	Hysterectomy
Shaw	18 days	Hysterectomy
H. 712 (Camb.)	23 days	P.M.
H. 710 (Camb.)	26 days	Hysterectomy
100 (CX.)	10 mm.	Hysterectomy
H. 33 (Camb.)	10 mm.	P.M.
H. 757 (Camb.)	10 mm.	Abortion
101 (CX.)	10 mm.	Hysterectomy
H. 186B (Camb.)	14 mm.	P.M.
102 (CX.)	15 mm.	Hysterectomy
103 (CX.)	22 mm.	Hysterectomy
H. 594 (Camb.)	22 mm.	Hysterectomy
H. 767 (Camb.)	22 mm.	Hysterectomy
104 (CX.)	25 mm.	Hysterectomy
H. 719A (Camb.)	27·5 mm.	Hysterectomy
105 (CX.)	29 mm.	P.M.
H. 630 (Camb.)	30 mm.	Hysterectomy
106 (CX.)	10 weeks (embryo not available)	Hysterectomy
107 (CX.)	31 mm.	Hysterectomy
H. 808 (Camb.)	33 mm.	Abortion
H. 643 (Camb.)	33·5 mm.	Hysterectomy
108 (CX.)	34 mm.	Hysterectomy
H. 789 (Camb.)	36 mm.	Hysterectomy
109 (CX.)	40 mm.	Hysterectomy
110 (CX.)	42 mm.	P.M.
H. 223 (Camb.)	42 mm.	Hysterectomy
H. 802 (Camb.)	45 mm.	Hysterotomy
H. 653 (Camb.)	46 mm.	Hysterectomy
H. 220 (Camb.)	47 mm.	Hysterectomy
H. 721 (Camb.)	48 mm.	Hysterectomy
111 (CX.)	50 mm.	Hysterectomy
H. 691 (Camb.)	59 mm.	Hysterectomy
112 (CX.)	60 mm.	Hysterectomy
113 (CX.)	60 mm.	Hysterectomy
H. 201 (Camb.)	60 mm.	Hysterectomy

The next available stage of development is an embryo presented by Dr G. A. K. Missen (late of the Post-Graduate Medical School and now of Guy's Hospital Medical School, London). The patient's menstrual history had been irregular for the previous 3 months. The last menstrual period began on 19 July 1953; the curettage was performed on 21 August. The part of the endometrium in which the embryo was subsequently found was fixed immediately in formol-sublimate saline. The block of tissue was cut serially at 5μ. Owing to the irregularity of the previous menstrual cycles and lack of detail on coital history, it is not possible to determine the precise age of this embryo. The maximum diameters of the chorionic sac (measuring from the edge of the trophoblastic shell) are 1·66 × 1·43 mm. The embryonic disc, which is cut obliquely, measures 0·280 × 0·214 mm. The primitive streak is differentiating and the first indication of Hensen's node and the primordium of the head process have appeared. The specimen, therefore, corresponds in state of development to such early embryos as Carnegie 1801 (Heuser, Rock & Hertig, 1945) and the Edwards–Jones–Brewer (Brewer, 1937, 1938); these embryos fall into the period of 13½–15 days of development. Through the kindness of Prof. T. B. Johnston of Guy's Hospital we had access to the implanted embryo, H. R. 1, described by him (Johnston, 1940, 1941). Prof. R. J. Keller of Edinburgh presented us with a single section through an implanted ovum obtained by curettage. The two specimens probably also fall within the same age-group.

The 'Gar' embryo, described by the late Prof. C. M. West, has been loaned to us by Prof. J. S. Baxter, of Cardiff. This embryo was obtained as a result of hysterectomy. From the menstrual and coital history, West (1952) concluded that the embryo was 18 days old. The developmental state of this specimen corresponds closely to that of other embryos known to be of this age. The chorionic sac was embedded in the anterior uterine wall close to the ostium of the left uterine tube. It appeared as a hemispherical haemorrhagic swelling 5 mm. in diameter and raised 4 mm. above the surface of the adjacent endometrium. A block of uterine wall including the myometrium and endometrium in which the embryo was embedded, was removed and sectioned serially. In the sections the chorionic sac is somewhat triangular in shape, tapering to a blunt point towards the myometrium. The widest internal measurement of the sac is 2 mm. The external measurement at this level is 4·1 mm. The embryonic disc measures 0·56 mm. antero-posteriorly and 0·6 mm. from side to side.

The next older specimen, the Shaw embryo, full details of which have been given in an earlier publication (Hamilton & Gladstone, 1942), was originally estimated from the coital history to be 20–23 days old. From its general development, however, we consider that its age is distinctly younger than this estimation and that it is about 18 days old. The uterus of this specimen was opened immediately after a subtotal hysterectomy. A small elevation about 1 cm. in diameter was observed on the posterior uterine wall. This swelling was excised with a block of surrounding tissue and fixed in Carnoy-sublimate. A complete series of sections, 10μ thick, was obtained. The measurements of the chorionic sac are as follows: maximum external diameter, 11 mm.; maximum internal diameter, 8 mm. The embryonic disc measured 1·05 × 1·34 mm.

The next specimen (H. 712) was an implantation site in a uterus removed for multiple fibromyomata. The history is unprecise as there had been menstrual irregularity for some years before the operation. The recorded L.M.P. was 3 months before the operation. The embryo, however, measured 1·8 mm. in total length and possessed seven somites. We place it at about the 23rd day of gestation. Unfortunately, the specimen only became available after the intact uterus, which had been in formalin for 8 days, was opened by a pathologist in the absence of knowledge of a possible pregnancy. Consequently, the implantation site is incomplete though the embryo itself was undamaged.

The last specimen (H. 710) on which specific comment is required is a complete implantation site from a uterus removed for menorrhagia. The menstrual history was irregular and it is not possible to give a precise date for ovulation. The coital history is also uncertain. The L.M.P. commenced on 14 December 1956 and the operation took place on 23 January 1957. Assuming ovulation on the 14th day of the cycle the ovulational age would be 26 days; comparison with the histories of the few other described somite embryos at comparable stages of development suggests that, in fact, H. 710 may be a little older than this. The uterus was opened immediately after operation. A pronounced swelling projected from the posterior wall of the uterus near the fundus. Half-strength Bouin's fluid was dropped on the swelling and a cruciate incision was made in the decidua capsularis to remove the embryo. A block of tissue containing the collapsed chorionic sac together with the underlying endometrium and uterine musculature was removed, embedded, and cut in serial sections. One half of the block was sectioned at right-angles to the surface of the endometrium and the other parallel to it.

The histories of the patients from whom the placentae associated with embryos of between the 10 and 60 mm. stages were obtained do not require special comment. The salient points of the specimens are given in Table 1. Some of the specimens were fixed by perfusion either from the uterine vessels or by way of the umbilical cord. In several of the specimens indian ink in plasma was injected either by way of the uterine arteries or directly into the intervillous space. Representative specimens of this period are illustrated in Pl. 2, figs. 4 and 5, and in Pl. 3, figs. 6–8.

DESCRIPTION OF SPECIMENS

11- *to* 12-*day stage*

The Barnes embryo. This embryo is almost completely implanted, the site of entry being covered with a thin layer of endometrium though the flattened uterine epithelium has not extended entirely over it (Pl. 1, fig. 1). The regional differentiation of the trophoblast is not uniform. Both primitive syncytium and cytotrophoblast are present but no definite indications of transitional cell types between the two have been seen. In the abembryonic region, towards the uterine cavity, the blastocyst wall is thin and composed almost exclusively of cytotrophoblast with only a small amount of syncytium, and here there is no lacunar formation. Elsewhere, however, the main mass of trophoblast is clearly differentiated into a cytotrophoblastic component and a spun-out primitive syncytium in which the intercommunicating lacunae, containing a small number of maternal blood corpuscles, are well established (Pl. 4, fig. 9). A wax-plate reconstruction of the implantation site indicates that the majority of the lacunae are in free communication with each other. When compared with the maternal blood cells elsewhere in the sections those in the lacunar spaces stain very poorly; presumably some influence operating on them in the spaces has markedly diminished their tinctorial affinities. The primitive syncytium possesses a granular cytoplasm; where it is in contact with the maternal endometrial tissue the latter frequently shows marked oedema and appearances of degeneration (Pl. 4, fig. 10). Where the syncytium forms the boundary of the lacunae it is usually irregular with surface projections of vacuolated protoplasmic fronds and streamers. In some regions, however, there is the appearance of a not very well defined brush border. In no part of the primitive syncytium were any mitotic figures observed. No evidence for amitotic division was found but frequently syncytial nuclei are closely apposed or clumped together (Pl. 4, fig. 11), although long stretches of protoplasm may be devoid of them (Pl. 4, fig. 10). The syncytial nuclei vary considerably in size (Pl. 4, fig. 12). The cytotrophoblast completely covers the embryonic mesoderm, which is, in fact, separated from this covering by a space which is a fixation artefact. There are in addition, however, aggregates of cytotrophoblastic cells, particularly round the equator of the chorion, which project into the syncytium. These aggregates may represent regional proliferative activity of the cytotrophoblast though no sign of special mitotic activity is shown by them. The cytotrophoblastic proliferations are always covered by syncytium and are similar in appearance to those described in other embryos at this stage of development (Stieve, 1936; Dible & West, 1941; Hertig & Rock, 1941, 1944; Wilson, 1954). Such proliferations have often been regarded as forming the early primordia of the chorionic villi and they will be discussed later. It must be stressed that, at this stage, the presence of the lacunae within the syncytium gives the latter a sponge-like structure. Consequently, the system of lacunar spaces is primarily labyrinthine and, as there are no free villi, the term 'intervillous space' is not quite appropriate.

The endometrial stroma, immediately round the implantation site, shows the predecidual reaction, and is markedly oedematous. The endometrial cells themselves do not yet show the histological changes distinctive of later stages. The intercellular spaces are distended with a slightly eosinophilic exudate containing occasional blood cells. The uterine glands are tortuous and dilated with large amounts of secretion. Those adjacent to the implantation site are deflected aside by the proliferating trophoblast. Glands near and in contact with the trophoblast show degenerative changes. In some instances the glandular epithelium has disappeared at the point of contact with the trophoblast which thus comes to plug the glandular lumen (Pl. 4, figs. 9, 11). Maternal blood corpuscles are present in the lumina of many of the glands but the precise source of such blood cells is not apparent.

Round the implantation site, and especially on its deep surface, a number of thin-walled, intercommunicating sinusoids, lined with endothelium, are in free communication with the lacunar spaces. At the points of communication the primitive syncytium is directly continuous with the endothelium lining the sinusoids (Pl. 4, fig. 12). There is no evidence at these points of junction of endothelial reaction to the syncytium. The sinusoids can be traced to veins in the deeper part of the endometrium. Typical coiled arteries are found in the endometrium; they are surrounded by regions of specialized, non-oedematous, decidua. Several of the arteries approach and become intimately related to the implantation site. We have, however, been unable to satisfy ourselves that there is any open communication with the lacunar spaces. Many of the arteries appear to end blindly in the endometrium. No evidence of alterations in the walls of the coiled arteries was observed in this specimen.

14- *to* 18-*day stages*

The Missen embryo. The general arrangement of the trophoblast and related endometrium in this embryo is shown in Pl. 5, fig. 13. It will be seen that the cytotrophoblast has increased very considerably in amount when compared with the 12-day specimen. The trabeculae of peripheral syncytial trophoblast of the previous stage have been invaded by the cytotrophoblast or have been transformed partially into it; certainly the latter tissue now forms a more or less continuous trophoblastic shell round the conceptus. In this peripheral shell there are to be found many spaces of varying size and shape. Some of these spaces contain maternal blood, others possess variously shaped masses of syncytium which is often vacuolated and shows phagocytosis of maternal blood cells. The nuclei of the syncytial masses frequently possess extremely large eosinophilic nucleoli. The spaces in the trophoblastic shell communicate only infrequently with the main portion of the lacunar system. On the maternal side of the trophoblastic shell many strands of peripheral syncytium are present; they extend as streamers into the surrounding endometrium making contact with the sinusoids, the coiled arteries and the glands. The presence of these syncytial streamers makes the external aspect of the trophoblast irregular. On the embryonic side of the trophoblastic shell, many of the trabeculae ('villi'), separated by the dilated lacunae, can be traced to the chorionic plate. These trabeculae are mainly arranged radially and are covered by a thin layer of syncytium. They now possess a mesodermal core. At the periphery of those of the radial strands which reach the trophoblastic shell there is a concentration of small, darkly staining, cytotrophoblastic cells which may well be the original source of the existing shell and which are doubtless the source of subsequent additions to it. The appearance of cytotrophoblast and of mesoderm within the trabecular syncytium does not alter the fundamentally labyrinthine nature of the lacunar spaces of earlier stages. Not all of these trabeculae, however, reach the trophoblastic shell for a number of them arise from the chorionic plate, or even from the main trabeculae, to terminate as *free* villi. It is such finger-like proliferations of trophoblast, with the contained mesoderm, that lay the basis for the subsequent villous structure of the placenta. At this stage, however, the human placenta clearly presents a combination of labyrinthine and villous characters. It must be stressed that the main trabeculae, which we will call primary villous stems, were never free villi. From this stage forwards, however, it would be pedantic to avoid calling the lacunar system the intervillous space.

The endometrium adjacent to the trophoblast in this embryo shows a well marked decidual reaction. Portions of the uterine glands in the immediate vicinity of the trophoblast are eroded or destroyed. Other glands, situated more remotely, show degenerative changes and maternal blood in their distended cavities. The endothelially lined maternal sinusoids in the implantation region are dilated and filled with plasma and some maternal blood cells. At a number of points, especially in the basal region, the intervillous space communicates with the sinusoids by way of channels through the trophoblastic shell (Pl. 6, fig. 15). Some of these channels are in part lined by syncytium but many of them possess walls formed by cytotrophoblast alone. The sinusoids can be traced away from the implantation site to become continuous directly with endometrial veins. As it is only the superficial part of the endometrium that is available in this specimen few details can be provided for the coiled arteries. Several of them, however, could be traced through the endometrium adjacent to the trophoblastic shell and into the latter where they terminate blindly. None was found to enter directly into the intervillous space; indeed, none could be traced into the spaces in the cytotrophoblast. In the walls of several arteries in the decidua compacta, which were separated by several hundreds of microns from the trophoblastic shell, there were signs of degeneration. A number of cells of syncytial origin were found in close relation to these endometrial arteries.

In embryo H.R. 1 (Johnston, 1940, 1941) and in Prof. Keller's curettage specimen conditions very similar to those described above for the Missen implantation site were found. In both of these specimens, however, the mesodermal cores of the villi are less well developed and the syncytium has perhaps undergone less retrogression (Pl. 6, fig. 16). In neither of them could coiled arteries be traced into the intervillous space.

The Gar embryo. This embryo with its implantation site has been described in considerable detail by West (1952). Here we restrict observations on it to points directly relevant to placental development.

The chorionic villi in this specimen are of two types. There are, first, those which have free endings in the intervillous space and which take their origin from the chorionic plate or from other villi near to their chorionic attachment. Many of these villi are well developed and branch as often as three or four times. The second villous type includes those which extend from the chorionic plate to the trophoblastic shell with which they are continuous. Such primary villous stems are, at least in part, persistent portions of the original trabeculae of the earlier stages. Apart from the small syncytial sprouts, which are attached to each type, the villi possess a well-developed core of mesoderm. They are covered by syncytial trophoblast deep to which a layer of cytotrophoblastic cells (Langhan's cells) can be identified. Early embryonic blood vessels are now present in the mesenchymal cores. At the periphery of the primary villous stems, the cytotrophoblast has broken through the syncytial layer and, by lateral expansion and fusion, constitutes a thick trophoblastic shell (Pl. 7, fig. 19). In the shell there are many labyrinthine irregular spaces, the walls of which are formed for the most part of cytotrophoblast alone. In these spaces there are many maternal blood corpuscles and such elements can also be found lying between the loosely arranged cytotrophoblastic cells themselves. The peripheral cells of the trophoblastic shell are intimately intermingled with the decidua and it is impossible to define a distinct foetal-maternal junction. Syncytial streamers from the trophoblast are occasionally found amongst the decidual cells. The syncytium frequently shows active phagocytosis (Pl. 6, fig. 17). The decidua basalis in this specimen is oedematous and, adjacent to the implantation site, blood corpuscles in large numbers are found amongst the endometrial cells. Coiled arteries can be seen in the deeper parts of the endometrium. As they approach the implantation site the walls of these arteries show necrotic changes; none was, in fact, found to open directly into the cytotrophoblastic lacunar spaces, nor into the intervillous space. The arteries adjacent to the implantation site have apparently been pushed laterally by the expanding chorion; some of them can, however, be traced to the decidua capsularis.

Large venous sinusoids lie adjacent to the implantation site; they anastomose freely with each other and receive a number of communications from the lacunar labyrinth by way of clefts in the trophoblastic shell. The most conspicuous communications between the intervillous space and the maternal veins in the Gar specimen are situated round the equatorial region of the trophoblastic shell. The uterine glands in the region of the implantation site are frequently invaded by the trophoblast with resultant destruction of the glandular epithelium. Indeed, in some regions syncytium is found within the lumina of the glands (Pl. 6, fig. 18). In many places the glands are filled with maternal blood.

The Shaw embryo. This specimen has already been described in some detail (Hamilton & Gladstone, 1942). Part of the decidua capsularis had been damaged previous to fixation and there is some distortion in the region of the decidua basalis. Notwithstanding these deficiencies, the specimen shows certain features relevant to understanding the development of the villi and the trophoblast. On the deep aspect of the chorionic sac, the wall of the implantation cavity is formed by an incomplete trophoblastic shell in which spaces containing small masses of syncytium are present (see pl. 3, fig. 6, in Hamilton & Gladstone, 1942). On its embryonic side, the shell is continuous with the cytotrophoblastic cell columns proliferated from the tips of anchoring villi, some of which are presumably villous stems. Freely ending villi, similar to those found in the Gar embryo, are also present. Syncytiotrophoblast of varying thickness covers the cell columns and the intervillous aspect of the trophoblastic shell. The mesenchymal cores of the villi are in the form of a loose reticulum in which there are some, for the most part empty, endothelially lined vascular spaces. These spaces, which are the precursors of the foetal villous vessels, are best developed in the region of attachment of the connecting stalk to the chorionic plate.

A wide space, the basal sinus (which may, in this specimen, be partly artefact), separates the decidua basalis from the trophoblastic shell and communicates very freely with the intervillous space. Owing to the small amount of maternal tissue removed with the conceptus, it has not been possible to trace the endometrial arteries or to determine with accuracy their mode of termination.

Somite stages (20–30 *days*)

Embryo H. 712 (7 *somites, c.* 23 *days*). Only a portion of the implantation site of this embryo is available. The trophoblastic shell is thinner than in the earlier specimens but in parts it can be seen to possess spaces. Villi are well developed over the whole of the chorionic sac. In those villi which reach and fuse with the cytotrophoblast the average length is about 1·5 mm. The villi are more branched than heretofore and consequently the intervillous space possesses a more complicated structure. The glands in the decidua show signs of secretory activity. The available portions of the specimen, unfortunately, do not permit of comment on either the spiral arteries or the uterine veins.

Embryo H. 710 (27 *somites, c.* 26 *days*). The implantation site in this specimen is shown in Pl. 2, fig. 3, and in Pl. 5, fig. 14. The embryo was removed by way of a cruciate incision through the decidua capsularis. On the deep aspect of the chorion the main villi have an average length of about 1·75 mm. They are also present, although less well developed, in the region of the decidua capsularis. All of the villi are covered by a continuous band of syncytium deep to which a fully established Langhan's layer of cytotrophoblast is present (Pl. 7, fig. 20). Well-established blood vessels are found in many of the villous mesodermal cores. The intervillous space contains some maternal blood. Peripherally many of the villi are attached to the attenuated trophoblastic shell by columns of cytotrophoblast. There are also islands of cytotrophoblast scattered, in an apparently random fashion, throughout the villous tree. The trophoblastic shell shows many spaces (Pl. 7, fig. 20). Some of these communicate with each other and others can be seen to open into the adjacent intervillous space. The spaces often contain cellular debris, glandular secretion and, occasionally, maternal blood cells. Many of these spaces also contain masses of syncytium which are often markedly vacuolated. The cytotrophoblastic cells of the shell are loosely arranged; debris and maternal corpuscles can frequently be seen between these cells.

The peripheral margin of the trophoblastic shell is intimately related to the decidua compacta and it is difficult to establish the precise line of junction between the two tissues. With suitable staining (e.g. Masson's trichrome technique or reticulin impregnation), however, it is always possible to identify the junction between maternal and foetal cells. The decidual cells are surrounded by collagenous and reticular fibrils; the cytotrophoblastic cells, however, have no fibrillar material between them (Pl. 7, fig. 21). Apart from some stray strands of decidual collagen which are apparently being digested by the cytotrophoblastic cells there are no intercellular fibrils in the trophoblastic shell. In the decidua basalis there are many dilated glands, often with large amounts of maternal blood in their lumina. Those in the marginal zone of the implantation site are distorted as they pass to their openings situated on the decidua capsularis (Pl. 5, fig. 14). The glands situated directly below the implantation, in the decidua basalis, have had their connexions with the uterine lumen interrupted. They terminate blindly in the junctional zone and many of them have their cavities invaded by cytotrophoblast. The secretion of such glands can in some instances be traced into the interstices between the cytotrophoblastic cells. Frequently, however, the appearance is presented of the damming back of secretion owing to the pressure exerted by the expanding conceptus. In those glands which flank the lateral margins of the implantation site there are frequently marked signs of degeneration. That part of a gland wall closer to the trophoblast shows cellular destruction while the opposite wall may possess a lining of intact glandular epithelial cells. (Illustrations of the relationship of the glands to the trophoblast can be found in Boyd, 1959.)

Many coiled arteries are found in the decidua adjacent to the implantation site. These vessels can be traced through the junctional region to the peripheral cytotrophoblast where most of them terminate, apparently blindly. Some of the arteries, however, have open endings which give the appearance of having permitted the escape of maternal blood into the interstices between the cells of the trophoblastic shell. None of the arteries, in spite of most careful search, could be found to discharge directly into the intervillous space itself. The walls of the terminal arteries show signs of degeneration with disappearance of their muscle cells and marked swelling of their endothelium (Pl. 7, fig. 22). Such striking changes in the endothelium can be found in loops of the coiled arteries quite remote from the junctional zone.

Openings from the intervillous space to endometrial veins are present over the basal surface of the implantation site; several large communications drain its equatorial region.

Specimens of in situ *placentae with embryos of the* 10 *mm. stage* (37–38 *days*)

Implantation sites of four embryos at this stage of development are available (Table 1). The general features of the utero-placental junction and of the chorionic villi are very similar in all four specimens. A photomicrograph (Pl. 8, fig. 23) of a section through one of them shows that, in comparison with earlier stages, the decidua basalis is now distinctly reduced. The decidua parietalis, however, is if anything even thicker than in younger specimens. The trophoblastic shell varies in thickness. Over extensive areas of the basal plate it has become attenuated; deep to such areas there is a fibrinoid deposit in the most superficial part of the decidua. In some regions this fibrinoid material shows a lymphocytic invasion. The shell still remains thick, however, where the well-developed cellular columns at the tips of the main villous stems are attached. No clear distinction can, in fact, be made, in such regions, between the cytotrophoblastic cells of the shell and those of the villous extremities. Between such cytotrophoblastic cells there are many of the spaces to which attention has already been drawn. Some of these spaces are in communication with the intervillous space, but most of them are isolated small cavities. Villi, both attaching and free, are distributed over the entire surface of the chorion. Opposite to the decidua basalis they have a slightly greater average length and are more luxuriant and better vascularized than those in relation to the capsularis. The cytoplasm of the trophoblastic components of the basal villi is generally more basophilic than that of the equivalent cells in the capsular villi.

Aggregations of cytotrophoblastic cells, in the form of the so-called cytotrophoblastic islands, are present in relation to the villi. Such islands are not restricted to, but are much larger on, the basal side. These islands will become increasingly apparent in later stages. Their superficial cells are in direct contact with the contents of the intervillous space.

The glands in the decidua basalis are very much distorted and the long axes of most of them are now orientated at an angle to the myometrium. They still show signs of secretory activity, however, and their lumina are frequently distended. Evidence of glandular breakdown in the neighbourhood of the implantation site is still apparent. There are many coiled arteries in the decidua and those in the basal part can be traced into the junctional zone. The walls of the dilated terminal parts of these vessels show a very marked fibrinoid degeneration with complete disappearance of the histological features of an artery. Some of these terminal arterial segments extend up to the trophoblastic shell and here the continuity of the vessel wall is often interrupted. Blood from these arteries and secretion and debris from the glands can be traced through gaps in the peripheral part of the shell to the cytotrophoblastic lacunae and, thence, to the intervillous space. Communications from the intervillous space to the endometrial veins are evident over the whole of the basal plate but, in one of the specimens, there is a preferential drainage from the zone of transition between the decidua basalis and decidua capsularis (Pl. 8, fig. 23). When the uterus of this 10 mm. specimen was opened, it was seen that the decidua capsularis projected across the uterine lumen and made contact with the decidua parietalis of the opposing wall. Here there was no fusion of the two deciduae but, as subsequent section showed, the epithelium of the decidua parietalis in the area of contact had become markedly thinned and the underlying endometrial capillaries and veins were markedly dilated.

Specimen of an in situ *placenta with an embryo at the* 15 *mm. stage* (41–43 *days*)

A section through the central part of the implantation site of this specimen is shown in Pl. 8, fig. 24. The diameter of the actual attachment area measures 26 mm. The thickness of the placenta proper varies between 3 and 6 mm. The combined thickness of the chorion and decidua capsularis is about 2 mm. and there are well-developed capsular villi. By this stage there is a striking reduction in the thickness of the decidua basalis but its glands continue to show secretory activity. Many of them open directly into the intervillous space and it is noteworthy that these are, for the most part, empty. Other glands, however, which do not communicate with the space, are distended with secretion; their cells are flattened and a number of them contain maternal blood. Except at the site of attachment of the cytotrophoblastic columns at the villous tips the thickness of the trophoblastic shell has become much reduced, often to a single layer of cells. On the decidual side of the shell there is frequently a fibrinoid deposit which is scattered in an irregular fashion over the surface of the basal plate.

Numerous arteries ensheathed by decidua can be traced from the myometrium towards the intervillous space. The terminal portions of these vessels show the hyaline degenerative changes described for earlier specimens and all of them are being invaded by cytotrophoblast. There is also a marked thickening of their endothelial linings in the deeper part of the decidua. The actual terminations of the arteries are in communication with spaces in the thickened parts of the trophoblastic shell. No direct arterial communications with the intervillous space itself have been observed. It would appear, therefore, that, at this stage, blood from these arteries could only have reached the intervillous space by percolation through the interstices situated between the cytotrophoblastic cells (Pl. 12, fig. 31). A striking feature of this specimen is the paucity of maternal veins underlying the central area of the implantation site. Consequently the main drainage of the intervillous space is through communications which open into veins in the region of junction of the decidua capsularis with the decidua parietalis. The villi themselves are clothed by both syncytium and cytotrophoblast; their mesodermal cores contain well differentiated foetal blood vessels.

Specimens of in situ *placentae with embryos of between* 20 *and* 30 *mm. stages* (48–60 *days*)

Sections through the whole uterus showing the developing placenta of the 29 mm. stage and of the 30 mm. stage are shown in Pl. 9, fig. 25, and Pl. 10, fig. 28, respectively. The placentae of these two specimens have diameters of approximately 40 and 47 mm. respectively. It will be seen from these illustrations that there are villi on the part of the chorion related to the decidua capsularis. The latter is now projecting deeply into the uterine lumen but has not yet fused with the decidua parietalis. Indeed, as will be seen from the account of later stages, such fusion does not occur until after the period of development with which we are concerned in this contribution. The trophoblastic shell is now very thin except in the regions of attachment of the terminal cytotrophoblastic cell columns of the anchoring villi. Extensions of syncytium into the decidua basalis are a striking feature of these stages. Such extensions frequently reach the myometrium and isolated syncytial masses can be found in its inner part. Other irregularly shaped plates of syncytium, often highly vacuolated, are present along the basal plate, particularly in the regions near the attachment of the villi.

The villi possess a structure similar to that of earlier stages. They are, however, more branched with resulting increase in the complexity of the intervillous space. The connective tissue in the villi is further differentiated and numerous Hofbauer cells are present.

As in previous stages, the walls of the terminal parts of the spiral arteries show fibrinoid and hyaline degenerative changes. Thickening of the endothelium is still present and in the portions of the vessels nearest to the intervillous space plugs of cytotrophoblast can be identified. Small irregular clefts can be followed from the arterial lumina through the interstices situated between the cytotrophoblastic cells. In some instances, indeed, as, for example, in a 22 mm. specimen, such clefts are represented by narrow, but straight, channels (Pl. 12, fig. 32) passing directly from the vessels to the intervillous space. Venous drainage from the intervillous space now occurs from point to point over the whole of the basal plate. By the end of this period of development centrally placed communications between the intervillous space and the decidual veins are numerous and often very large (Pl. 11, fig. 30).

Specimens of in situ *placentae with embryos of between the* 31 *and* 60 *mm. stages* (60–90 *days*)

We have sixteen specimens of *in situ* placentae which, on maternal history and foetal size, can be considered to belong to the third month of gestation (Pl. 9, figs, 26, 27; Pl. 11, fig. 29). The shape of these placentae is variable but the average transverse diameters range from 50 to 70 mm. There is also considerable variation in placental thickness from specimen to specimen and even in different regions in a given placenta. Although the chorionic sac becomes much larger during this period the increasingly thinned and stretched decidua capsularis has not, in any of the specimens, yet fused with the decidua parietalis. The villi related to the decidua capsularis, however, show marked signs of retrogression. This retrogression, in the earlier specimens, is most apparent at the pole opposite to the placenta. As older specimens are examined the disappearance of villi is seen gradually to spread from this pole in an equatorial direction so that by the end of the third month nearly the whole of the decidua capsularis is related to a part of the chorion in which the villi are represented only by little stumps. Accompanying the villous retrogression there is a disappearance of the associated intervillous space. The villi in the placenta itself become much more numerous in this period. The main villous stems become thicker and there are more numerous side branches. In the initial part of the third month the cytotrophoblast on most of the villous surfaces constitutes a continuous Langhan's layer. By the middle of the period, however, areas in which this layer cannot be identified by light microscopy have become apparent and in the oldest specimens it has disappeared in most of the villi.

The trophoblastic shell is very attenuated in all the specimens except in the area of villous attachments. In certain regions the shell may be reduced to a single layer of cells. Throughout the whole period fibrinoid material is distributed irregularly over the whole of the basal plate. Attached to the apical portions of the villi there are many syncytial sprouts. Some of these sprouts are also distributed from point to point throughout the villous tree. In some of the older specimens in this period there are projections from the basal plate into the intervillous space. These projections are the forerunners of the cotyledonary septa which become such important features in the placenta of the second half of pregnancy.

The decidua basalis is slowly but continuously reduced in thickness throughout the third month of gestation. Nevertheless, glandular activity can be detected in all of the specimens. A striking feature is marked development of the uterine glands in the decidua parietalis at the angle of junction of the decidua basalis with the decidua capsularis. Occasional haemorrhage into the basal glands can be detected in all of the specimens. Erosion of some of these glands by cytotrophoblast is also shown in most of them. The decidua basalis in every specimen contains many masses of multinucleated cytoplasm which, on the basis of orientation, distribution and cytology, we (1960, in press) consider to be of trophoblastic origin. Such syncytial masses are often arranged radially as they penetrate through the decidua to reach the inner part of the myometrium where they come to lie between the muscle cells.

Spiral arteries can be traced through the decidua to the cytotrophoblast. These arteries show the degenerative changes in their walls to which attention has been directed in the descriptions of the earlier stages. In all the specimens these arteries terminate in a mass of cytotrophoblast and cells of foetal origin can be traced up most of the vessels. In no specimen have we found spiral arteries to open freely into the intervillous space. There are always interposed plugs of cytotrophoblast. Such plugs become more loosely arranged as development proceeds and in the oldest specimens in this period (Pl. 12, fig. 35) they give the appearance of being much less capable of acting as an impediment to blood entering the intervillous space. Sections were available of a specimen into which indian ink had been injected into a uterine artery in the broad ligament before the uterus was opened. Such sections (Pl. 12, figs. 33, 34) show ink in the lumina of the spiral arteries from whence it can be traced through intercellular cytotrophoblastic spaces into the intervillous space. In all of the specimens of the third month uterine veins are found to communicate freely with the intervillous space over the whole of the basal plate (Pl. 12, fig. 36).

DISCUSSION

Endometrium and decidua

At the time of implantation of the blastocyst the human endometrium shows a distinct division into a superficial *stratum compactum* and a deep, oedematous, *stratum spongiosum*. It measures about 4–5 mm. (see Krafka, 1941, for details) in thickness and is, of course, under the influence of the luteal hormone secreted by the ovary. After implantation, the endometrial oedema becomes even more marked, particularly in the región underlying, and round, the blastocyst. The oedema, which is presumably to be attributed to alterations in the walls of the blood vessels or in the flow through them, results in an eosinophilic exudate which separates the endometrial cells from each other. The blood vessels near the implantation site are hyperaemic and sinusoidal vascular spaces become apparent; such spaces may be dilated venules or capillaries. Initially, there seems to be little effect on the spiral (coiling) arteries themselves, but the pre-decidual reaction is more intense round them. Wislocki & Streeter (1938), in their studies on implantation in the macaque, have described endometrial changes similar to those just indicated for the human endometrium; in the monkey, however, in the region of attachment of the blastocyst, there is a marked proliferation of the epithelium lining the uterine lumen and in the necks of the glands. This epithelial proliferation is presumably to be associated with the central method of implantation in the macaque.

In our 12-day human specimen the endometrial oedema is well marked. In view of the findings by Hertig *et al.* (1956) on a number of early specimens, this oedema must be regarded as a normal feature of the endometrium, though Davies (1944) mistakenly considered it to be pathological. Further, in well-fixed specimens such as the Barnes ovum, there is no infiltration of maternal small round cells into the endometrium. Such infiltration is marked in a number of specimens (e.g. Bryce & Teacher, 1908; Davies, 1944) which can, in our opinion, be definitely considered abnormal. Indeed, the absence of lymphocytes from the region surrounding the implantation sites is a striking feature of normal early specimens for it is quite unlike what would be expected if the trophoblast behaved immunogenically as a homograft (Boyd, 1959).

The stromal endometrial cells in the 12-day specimen cannot be regarded as more than pre-decidual in their degree of differentiation. By the 14th day, however, the characteristic and definitive decidual cells have become apparent; these cells persist throughout the stages of development dealt with in this contribution. The decidual cells can always be readily distinguished (Pl. 7, fig. 21) from the cytotrophoblastic cells by the fact that the former are surrounded by collagen and reticulin fibrils.

Uterine glands

In the luteal phase of the uterine cycle the endometrial glands become enormously enlarged. Attachment of an implanting conceptus normally occurs between openings of such glands on the uterine surface (Pl. 2, fig. 2). As the blastocyst becomes interstitially implanted the glands adjacent to it are compressed by the pressure of its circumferential growth. Consequently there is a damming back of contained secretions in the glands concerned and they become much dilated while their necks become stretched, compressed and, finally, occluded. Meanwhile, the actively proliferating trophoblast erodes the endometrial stroma and glandular epithelium. eventually breaking through into the glandular lumen (Pl. 6, fig. 18; and see Boyd, 1959, pls. 1, 2). In this manner the contents of the glands become directly available to the trophoblast (Pl. 4, figs. 9, 11). It is the glands in that part of the endometrium which will become the *decidua basalis* which are involved in the implantation process and which become dilated. The necks of some of them are also drawn into the lower (i.e. attached) part of the *decidua capsularis* but the glands in the *decidua* *parietalis* persist until a remarkably late stage (Pl. 8, fig. 23) in a relatively undistorted condition. As development proceeds, however, glands initially remote from the implantation site are gradually encroached on by the trophoblast. Thus, in the 12-day specimen only a few glands are affected, there are considerably more involved in the 17- to 18-day specimens, and by the 28th day (Pl. 5, fig. 14) a very great many, indeed, are included in the process. Gradually the glands in the basal decidua are, for the most part, destroyed. Owing to the nature of placental expansion, however, the glands at the margin of the placenta are much less affected than those in the central region; consequently, well-preserved glands can be found in the marginal zone of the decidua basalis throughout the period with which we are at present concerned. Glandular secretion is apparent in many of the persisting basal glands until comparatively late stages (Hamilton & Boyd, 1951).

Possibly as a result of the glandular penetration by the destructive action of the trophoblast, maternal blood cells are early apparent in many of the glands. Such extravasated blood is already present in several glands in the 12-day specimen (Pl. 1, fig. 1) and is a striking feature in the central part of the decidua basalis of the 28-day specimen (Pl. 5, fig. 14). From the accounts in the literature on early implantations it would seem that such haemorrhage into the glands does not occur earlier than the 12th day. Even in later stages it may be absent (for discussion, see Krafka, 1941). Blood in the glands, however, has been described in so many older specimens that its presence must be considered a normal feature of human implantation from 12 days onwards. Haemorrhage into the uterine glands has also been described in the neighbourhood of the implantation site in the macaque by Wislocki and Streeter (1938); they found that the blood was not present in the glands until the 17th post-ovulational day and that it persisted until the end of the first month of conception. Haemorrhage into glands which are not yet occluded by the pressure of the expanding trophoblast is probably the explanation for the slight external haemorrhage, in both women and the macaque monkey, now well known to occur at, or about, the time of the first 'missed' menstruation. This blood loss constitutes the so-called 'placental sign' to which attention was first directed by Hartman (1928, 1932).

Trophoblastic differentiation

Reference has been made in the introduction to certain difficulties in the terminology used to describe the different varieties of trophoblast. In particular, we have avoided terms such as 'implantation trophoblast' and 'resorptive syncytium' that attribute specific functions to parts of the trophoblast at successive periods of development. There can, of course, be no doubt that there is a functional specialization on the part of the different regions of the trophoblast; until more is known about the details of trophoblastic function, however, terms implying a particular activity by a given region should be avoided. Moreover, as Wislocki & Streeter (1938) have stated, such an adjective as 'resorptive' can apply 'equally well to the entire development of the trophoblast and should not be restricted to any particular stage'.

In the $7\frac{1}{2}$-day human ovum described by Hertig & Rock (1945) the blastocystic trophoblast has already differentiated at the implantation pole into a thick plaque of proliferating, and intermingled, syncytium and cytotrophoblast. The remaining part of the blastocyst wall is constituted by a thin 'mesothelial' membrane which consists of a single layer of flattened blastocystic cells. In the junctional region between the latter and the plaque itself cells intermediate in character between the two are found. In specimens several days older (Hertig & Rock, 1945, 1949), when implantation is nearly achieved, differentiation into primitive cytotrophoblast and primitive syncytiotrophoblast is much clearer. Both of these are alike derived from the blastocystic trophoblast but the syncytium arises indirectly in the sense that it stems from cells which are uninucleate and which, consequently, cannot initially be distinguished from other cytotrophoblastic cells. How the syncytium actually takes its origin is an important question to which, so far, there is no compelling answer. Either certain cytotrophoblastic cells undergo nuclear division—mitotic or otherwise—without cellular division, or adjacent cytotrophoblastic cells unite and, by their fusion, constitute a multinucleate mass of cytoplasm. We have given much attention, throughout the study of all of our material, to the method of syncytial formation in the trophoblast and have been unable to decide between the two possibilities. The syncytium presents a histological structure quite strikingly different from the cytotrophoblast. Hence, if elements of the latter are included by fusion in the former the cytotrophoblastic cytoplasm must rapidly change in character during the assimilation. On the other hand, if there is nuclear multiplication in the syncytium, the absence of signs of mitosis in the multinucleate cytoplasm suggests the not very satisfactory explanation of amitotic division. Whatever factors do, in fact, cause the appearance of the syncytium, contact with the endometrial tissues does not seem to be an absolutely determining one, for primitive cytotrophoblast is found in direct apposition to the maternal cells. Once it has appeared, however, the primitive syncytium seems to be mainly responsible for the local destruction of the maternal tissues.

Soon after its first appearance the syncytium of the trophoblast shows marked vacuolation. The vacuoles become confluent and by the 12th day a well-established system of lacunar spaces is present throughout the syncytium. Moreover, some of the dilated maternal sinusoids are now in open communication with the lacunae and maternal blood is present in them. The method whereby the sinusoids are tapped is not apparent. It does not appear to be a disordered destruction of maternal tissue that is responsible; the endothelium of the maternal vessels, at the points of communication, is in neat apposition to the peripheral syncytium (Pl. 4, figs. 9, 12), and there is no sign of maternal haemorrhage into the decidua surrounding this part of the trophoblast. The maternal blood cells in the lacunar spaces in the 12-day specimen are very palely stained. The difference in the degree of eosinophilia between them and the maternal erythrocytes in blood vessels away from the implantation site is quite striking. It is, therefore, possible that the cells in the lacunar spaces in this stage, due to the stagnant nature of the circulation or to the effect of syncytial secretion on them, are in a special physiological state.

In the 12-day specimen the primitive syncytium covers the cytotrophoblast and the latter is present only in some of the trabecular columns which incompletely separate the lacunae from each other (Pl. 1, fig. 1). There is no sign, at this stage, of villus formation by the extension of separate syncytial 'streamers' into a maternal blood space. The traditional picture of 'primary villi' is one that must be dismissed.

It was based on conclusions arrived at from the study of badly fixed material and on an absence of understanding of the method of formation of the lacunar spaces and of the relationship of the trophoblast to them. Already at the 12th day the human placental arrangement, to use Grosser's terminology, is haemo-chorial, but at this stage it quite obviously possesses not a villous structure but a labyrinthine one.

In the period from the 14th to the 20th day there is intense proliferative activity in the trophoblast; this activity results in the appearance of the definitive structure of the placenta and in the full establishment of the circulation in the rearranged lacunar spaces which now, collectively, constitute the intervillous space. The first result of the growth processes is that the trophoblastic trabeculae, instead of being arranged quite irregularly, tend, more and more, to become orientated radially round the chorion and, therefore, to take on a villous appearance. Soon these trabeculae, which initially are syncytial in character, come to possess a cellular core by active multiplication of the trophoblastic cells on their deep, or chorionic, surface. It is at this stage that the now highly modified trabeculae have been called 'primary villi'. It must again be stressed, however, that the primary villous stems, as we have called them earlier, do not arise as individual and separate sprouts from the chorion. With continuing development the primary villous stems increase in length and, concomitantly, their cytotrophoblastic cores extend distally to the region of attachment of the syncytium to the endometrium. Coincidental with the extension of the cytotrophoblast the stems, at their chorionic ends, develop mesodermal cores which follow the cytotrophoblast in its extension towards the decidua. In part, this mesoderm in the villous stems is the result of proliferative activity in the general extraembryonic somatopleuric mesoderm of the chorion. The mesodermal cores, however, seem also to receive contributions of cells derived from the cytotrophoblast in the manner first described by Hertig (1935). Whatever the actual origin of the mesoderm in the villous stems, once it has appeared the stage of the 'secondary villi' of earlier authors has been established. The newly formed mesodermal cells of the cores of the villi do not come into direct contact with the syncytium on their surfaces. Proximally (i.e. at their chorionic ends) the villous stems preserve a single layer of cytotrophoblastic cells which, as Langhan's layer, separates the mesodermal core from the overlying definitive syncytium. Distally the cytotrophoblastic cells, as the cytotrophoblastic columns, form the full thickness of each villous stem. On their free surfaces the columns are covered by definitive syncytium which separates them from the enlarging intervillous space. At their decidual ends, however, active proliferation of the cytotrophoblastic columns results in an expansion of the cytotrophoblast beyond the distal ends of the villous stems where their cells extend into the primitive syncytium. In this position they mushroom outwards from the maternal end of each villous stem with the result that, eventually, they meet equivalent cells from adjacent primary villous stems to form an irregularly complete cytotrophoblastic layer, known as the trophoblastic shell, round the whole conceptus. This shell was first described and named by Siegenbeek van Heukelom (1898) and his description was amplified by Peters (1899). The term is a useful one for it ascribes to the expanded and fused tips of the primary villous stems precisely that feature which is most characteristic of the resulting cytotrophoblastic layer—that it forms a shell for the developing conceptus and placenta.

Any decidual cells included within the trophoblastic shell are soon destroyed, for our own observations confirm those of Jones & Brewer (1935) and Brewer (1937) that no maternal cells persist within it. Immediately on the decidual side of the trophoblastic shell there is the special transformation of the endometrial stroma to which Merrtens (1894) first directed attention, and which has variously been known as the 'boundary zone', the *Durchdringungszone*, *Umlagerungszone* and the 'trophosphere'. It is the compact decidua, immediately surrounding the trophoblastic shell, which presents a different histological picture from the more remotely situated endometrium. In it are the terminations of the maternal blood vessels and through it the blood must pass to reach the intervillous space. In the region of junction between the boundary zone and the shell there appears the fibrinoid deposit which is called Nitabuch's membrane and which is shown in our 28-somite stage and in all of the older specimens (Pl. 12, figs. 33–36). The boundary zone is traversed by the maternal blood vessels on their way to and from the intervillous space and, in the younger stages, uterine glands pass through it to open into spaces in the trophoblastic shell or even into the intervillous space itself (Frassi, 1908; Boyd, 1959).

By the method of its formation the trophoblastic shell splits, in an irregular fashion, the initial primitive syncytium into a part on the intervillous space aspect of the shell and a part on its decidual aspect. The former of these two portions of the primitive syncytium becomes the lining of that part of the intervillous space in contact with the basal plate of the placenta. The residual syncytium on the decidual side of the shell persists only in part and in an irregularly distributed fashion until late developmental stages; this persisting peripheral syncytium probably gives origin to some of the giant cells of foetal origin which become so widely distributed in the decidua basalis and the adjacent part of the myometrium (Boyd & Hamilton, 1960). The cells of the trophoblastic shell itself, however, also appear to be able to differentiate into syncytial giant cells. Indeed, after it has apparently reached a stage of full differentiation the cytotrophoblast retains the potency to produce syncytium throughout pregnancy.

The trophoblastic shell, though in general consisting of closely packed cells (Pl. 7, fig. 21), possesses many variously shaped, and occasionally quite large, intercellular spaces and gaps to which attention was first drawn by Jung (1908). Many of these spaces come to communicate with the peripheral part of the intervillous space, and some of them do so with the lumina of the uterine glands. In the spaces (Pl. 7, fig. 20) detritus, glandular secretion and, eventually, maternal blood cells are found, often accompanied by portions of syncytium. In our opinion these spaces are of considerable importance in the earlier histiotrophic phase of embryonic nutrition and they are also important in that many of the endometrial spiral arteries will communicate with the intervillous space by way of them. It must be stressed that the cells of the cytotrophoblastic shell never develop mesenchymatous derivatives equivalent to the mesodermal cores of the villous stems and the villi. Hence, when the foetal blood vessels develop in the mesenchymal cores to produce the 'tertiary villi', they do not penetrate the shell and do not come into contact with the maternal decidua or blood.

By the establishment of the trophoblastic shell a mechanism is apparently provided, through its interstitial growth, for a rapid circumferential extension of the whole implantation site. The surrounding decidua, including the glands, is encroached upon and, at the same time, the side of the implantation site in contact with the decidua capsularis bulges out into the uterine lumen. Generally the cytotrophoblastic shell becomes markedly thinned from the 10 mm. stage onwards, and, at the same time, the related fibrinoid material increases in amount. In the regions overlying the attachment of the villi, however, its attenuation is much less marked.

As a result of the expansion of the trophoblastic shell the area available for the intervillous space is considerably augmented. Into this enlarged area extend sprouts from the surface of the primary villous stems and also, we think, from the surface of the chorion itself. These sprouts are initially formed of definitive syncytium alone. Soon, however, they increase in size and pass through the stages of villi possessing cytotrophoblastic, mesodermal and, eventually, vascularized mesenchymal cores. Such sprouts are undoubtedly true villi and their presence justifies the ascription of a villous character to the human placenta. Nevertheless, these true villi are a secondary addition to the structure of the placenta. Some of them later become joined together at their free ends by syncytial fusion, but we doubt if this fusion involves more than the syncytium for we have never observed unequivocally the junction, described by Stieve (1941), of separate villi by the fusion of their mesodermal cores or of their constituent blood vessels. We have not, by direct observation, been able to decide if some of the villi arising from the chorionic plate manage to reach the basal plate and to fuse with it. As the placenta grows extremely rapidly in the period between the 5 mm. stage and the 60 mm. stage it seems likely that new major villous stems are continuously added. There may, however, be some longitudinal splitting of the initial primary villous stems. Certainly there is rapid growth in their length and marked branching at their basal extremities.

The earliest specimen available to us, the Barnes ovum, possesses a system of well-developed lacunae in the trophoblastic syncytium (Pl. 1, fig. 1). Several sinusoids communicate with the lacunae (Pl. 4, figs. 9, 12) in which, as has already been stated, maternal blood cells are present. Because of their size we presume that these sinusoids have arisen from endometrial venules; they are too large for a capillary origin to be likely. The sinusoids can be traced away from the implantation site for long distances to become continuous with the basal endometrial venous plexus. Several spiral endometrial arteries are present in the vicinity of the implantation site in the Barnes specimen, but no connexions between them and the lacunar spaces have been found. Hertig & Rock (1941), in their description of a human implantation of the 11th day, have described the related sinusoids as being arterio-venous in nature, considering that there is an arterio-venous anastomotic connexion between an adjacent spiral artery and the sinusoids. We have been unable to identify any such anastomoses, and the work of Bartelmez (1957) makes their presence unlikely. It seems to us, therefore, that the first maternal vessels to be 'tapped' by the lacunae are slightly dilated venules or capillaries in the stratum compactum of the decidua. With further extension of the invading syncytium, and more dilatation of the blood vessels, the supply of blood to the lacunae comes from venous sinusoids. That no direct arterial openings into the intervillous space are found in presomite embryos, such as the Edwards-Jones-Brewer (Brewer, 1937), the HR 1 (Johnston, 1940), the Shaw (Hamilton & Gladstone, 1942), the Torpin (Krafka, 1941) and the Gar (West, 1952), would seem to indicate that the flow of blood into the spaces is a 'controlled seepage', as Krafka has suggested. It can further be pointed out that, in the macaque monkey (Wislocki & Streeter, 1938), it is not until the 17th day that maternal vessels discharge their contents into the lacunae. A continuous flow of blood through the intervillous space was not considered likely until the 3rd week. These investigators, however, go further for they state: 'We have no absolute information as to when the maternal blood actually begins to flow through the intervillous space, but it may safely be assumed as occurring gradually and as probably being completed in the main before the time that the fetal circulation is established.'

Whether the blood in the syncytial lacunae is simply 'pooled' or is subject to a slow ebb and flow circulation cannot be decided on the evidence. We have already recorded, however, that the maternal blood cells in the lacunae of the 12-day specimen show staining characteristics suggesting that the circulation is, in fact, very sluggish. Mossman (1956) has suggested that, as the perforation into a single sinusoid (he actually instances a capillary) elongates, two openings, an inlet and an outlet, can be achieved. With increase in the trophoblastic invasion, Mossman considers that the maternal vessels would disappear progressively back to the level of true arterioles and venules, thus permitting a 'through' circulation.

Whatever the explanation of the precise method whereby the spiral arteries themselves come to open into the intervillous space we have not been able to find communications of the arteries with the space until the 10 mm. stage. Even at this stage the communications are only by way of gaps in the trophoblastic shell. Doubtless capillaries have been tapped long before this stage and as their blood pressure is presumably higher than that of the veins a proper circulation through the intervillous space then becomes possible. In our 27-somite specimen, for example, the probability that such a circulation existed in life seems most likely. Indeed, Ortmann (1938) has described spiral arteries opening directly into the intervillous space in a 9-somite embryo originally described by Veit & Esch (1922). In our well-fixed 28-somite specimen, while we have found appearances very similar to some of those illustrated by Ortmann, we have been unable to convince ourselves that the spiral arteries themselves actually open into the intervillous space. But whether they do or do not so communicate already in the 28-somite specimen, the walls of the terminal portions of the spiral arteries show marked alteration and, in particular, a marked hypertrophy of their endothelial linings (Pl. 7, fig. 22). In subsequent stages the changes in the walls of the arteries become much more marked so that, for long stretches of their length, back from the implantation site, they show a striking degenerative appearance, with disappearance of their muscle cells, hyaline alterations in their walls, and dilatation of the terminal parts of their lumina. Further, from the period when the spiral arteries can actually be found to communicate with the intervillous space, there is the appearance of cells within their lumina (Boyd & Hamilton, 1956). Some of these intrusive cells are certainly of foetal origin for they possess the same histological characteristics as the cytotrophoblast and, indeed, can be traced directly from the shell into the arteries (Pl. 12, figs. 31, 35). It is possible that some of the intravascular cells in the more proximal part of the spiral arteries are of maternal origin, being derived from the hypertrophied endothelium, the muscle cells, or, even, migrating elements from the adjacent decidua. Our strong impression, however, is that most of the intravascular intrusive cells are of trophoblastic origin.

Up to the 43 mm. stage such cells are found in all of the arteries that pass through the transitional zone and they are also present in many of these vessels until much later stages (see illustrations in Boyd, 1956). The presence of these cells within the spiral arteries during human pregnancy has been recorded by a number of investigators (for summary, see Grosser, 1927). Similar intravascular cells have been reported in the pregnant uteri of other mammals (for summary, see Orsini, 1954). The discussion in the literature has chiefly related to the possible origin of these intra-arterial cells; little consideration has been given to their possible functional import. The constancy with which they are present in the human subject seems to us to justify the conclusion that they play some part in the placental mechanism, probably by cutting down very considerably the pressure in the blood which reaches the intervillous space. In several of our specimens in which we injected indian ink into the main uterine arteries, subsequent histological examination of the placentae showed that the ink passed from the spiral arteries through the restricted spaces between the intravascular cells and then by way of very narrow chinks in the cytotrophoblast before reaching the intervillous space itself (Pl. 12, figs. 33, 34). Under such conditions the pressure of the blood reaching the intervillous space must be very much reduced. The extension of the trophoblast into the arteries is obviously not due merely to the growth of this foetal tissue. The invasion of the arteries is selective for it can be affirmed quite dogmatically that, in the human placenta, the veins draining from the intervillous space never show such an invasion.

As has been stated previously, the sinusoids draining the early trophoblastic lacunae can readily be traced to the basal endometrial veins. As development continues the sinusoids increase in number and, indeed, cannot be distinguished from veins. In our earlier specimens the veins are found draining at irregular intervals from the whole of the basal surface of the developing placenta. Ortmann (1938), in his somite specimen, describes and figures the venous drainage as being exclusively from the central part of the basal plate. In our somite specimens, however, the drainage is both central and from the margin. As development proceeds the central veins are retained (Pl. 11, fig. 30) and are progressively added to in number as the placenta increases in size. In none of our placentae up to the 60 mm. stage is there an exclusive venous drainage from the marginal placental zone.

From our observations on the arterial supply to, and the venous drainage from, the intervillous space in the period with which we are at present directly concerned it seems to us justifiable to conclude that the circulation of maternal blood through the early placenta is under conditions which suggest that the pressure of the blood is low and its rate of flow slow. Moreover, as the maternal arteries and veins are connected with the intervillous space at random over the whole of the basal plate, the circulation in the space cannot be one that follows a very fixed path. There may, perhaps, be some preferential channels through the intervillous space but our attempts to establish their presence have been quite unsuccessful. This overall picture of blood entering and leaving the intervillous space at random is not very satisfying, particularly if one is looking for an optimum method for gaseous exchange between the maternal and the foetal organism. Nevertheless, we consider that it corresponds with what is to be found from an unprejudiced survey of the *in situ* placenta.

In an earlier communication (Hamilton & Boyd, 1950), based on a study of such human placentae, we indicated that a re-assessment of the circulation within the intervillous space seemed to us to be necessary. Until the contribution of Spanner (1935) the generally accepted view on this circulation was the one put forward by Bumm (1893) who considered that, in the mature placenta, the orifices of the endometrial arteries into the intervillous space are situated in the placental septa some distance from the basal plate and that the openings of the uterine veins draining the space are through the basal plate about the middle of each cotyledon. This view was questioned by Kermauner (1912). More recently Ramsey (1956*b*, 1959), in a series of papers on the macaque placenta, using modern techniques and with abundant material has shown that, in fact, the arterial openings are independent of the septa. She considers that what Bumm mistook for arteries in the septa were artefacts, being the remnants of glands, lymphatic channels or localized foci of necrosis. Bumm had postulated that the blood flowed back from the arterial openings in the septa, through the intervillous space, to the venous openings in the basal plate; he considered that, as a result, each cotyledon of the placenta functioned as a separate circulatory unit.

Spanner (1935), from his studies of the placenta after injection of the maternal circulation with celluloid and later digesting away the soft tissues, reached quite a different conclusion on the intervillous space circulation. He considered that the endometrial arteries only opened through the basal plate and that all the blood was drained away from the intervillous space through a constant, dilated, peripheral portion of this space which he designated the marginal sinus. In his view it was only at the placental margin, i.e. at the marginal sinus, that the intervillous space communicated with the uterine veins. In criticizing Spanner's theory of the circulation Ramsey (1956*a*, 1959) points out the possibility that, in Spanner's material, the injection medium occluded the slender, oblique central venous drainage channels while accumulating in certain wider more marginal channels. Whatever the explanation of Spanner's conclusions we can substantiate our earlier statement (Hamilton & Boyd, 1951) that at all stages from the late somite embryo until stages up to foetuses of 60 mm. (and, for that matter, until full term) there are, in the basal decidua, many centrally placed veins draining the intervillous space. We do not deny that, here and there, a vein may occasionally drain from the peripheral region of the intervillous space; indeed we have not infrequently observed such drainage. Nevertheless, we agree with Ramsey (1956*a*) that this marginal drainage in the human placenta can be relegated to an unimportant role in the explanation of the circulation in the intervillous space.

In the stages of placental development with which we are at present concerned the septa, which are such striking features of the mature placenta, have not yet become established. It is obvious, however, that the septa are only a secondary modification and that a fully functional circulation can exist in their absence. Their absence in the earlier stages removes, of course, real substance from Bumm's interpretation which demands the presence of the septa as an effective part of the circulatory mechanism. It also detracts strongly from Spanner's interpretation of the septa as baffles essential for ensuring that the blood from the openings of the arteries in the basal plate passes down to the chorionic plate, where, as the so-called subchorial lake, it is directed, for drainage, to the marginal sinus. Indeed, we consider that the course of the circulation in the mature intervillous space is probably much as we depict it diagrammatically in Pl. 13. Such a circulation would result when the septa are added to the arrangement we find present up to the stage of placental development in the 60 mm. C.R. length foetus. It also corresponds closely to Ramsey's (1956*a*, 1959) interpretation of placental circulation for both monkey and Man. It is supported by the observations on human placentae by Stieve (1941, 1942), Kladetsky-Haubrich (1952), Bøe (1953, 1954) and Browne (1954). To the circulatory mechanism existing in the placenta itself must be added, as Hinselmann (1925), Wagner (1929), Kladetsky-Haubrich (1952) and, particularly, Ramsey (1959) have pointed out, myometrial contractions; these may play an important role by causing intermittent elevations in the intervillous space pressure. When such Braxton-Hicks's contractions decrease, or cease, the blood from the space will then drain into the uterine veins. Ramsey has also suggested that the head of pressure in the arterial blood entering the intervillous space is sufficient to ensure that the blood is not short-circuited into the adjacent venous orifices. This mechanism may well operate in the fully established and mature placenta but can hardly do so when the arteries contain the plugs of cytotrophoblast to which we have drawn attention.

SUMMARY

1. A description is given of the development and differentiation of the trophoblast in Man from about the 12th day until about the 90th day (i.e. to a stage with an embryo of 60 mm.).

2. In the earliest specimen the primitive syncytium forms trabecular columns, which incompletely enclose separate lacunar spaces. At a slightly later stage of development these columns become radially arranged to form the primary villous stems.

3. The stems acquire cytotrophoblastic cores as a result of the proliferation of the cytotrophoblast of the chorion; at their chorionic ends, with the development of mesoderm, secondary villi are then produced. There are no 'free villi' at this stage and the developing placenta is essentially labyrinthine.

4. With continuing development the cytotrophoblastic cells at the peripheral extremities of the secondary villi proliferate to form cell columns; when these reach the primitive syncytium they mushroom out in it and fuse to form the trophoblastic shell.

5. Interstitial growth within the shell results in an increase in size of the implantation site.

6. The circulation in the lacunae is at first sluggish. The spiral arteries do not open directly into the intervillous space; the maternal blood percolates through intercellular gaps in the trophoblastic shell and leaves it through randomly distributed venous openings.

7. No arterio-venous anastomoses connecting the spiral arteries with the sinusoids have been identified.

8. When the maternal arteries come to communicate with the intervillous space they also randomly perforate the basal plate.

9. The arterial orifices into the intervillous space are invaded by cytotrophoblastic cells, and such cells can be found in these vessels throughout the remainder of the period of development under consideration.

10. The endothelium in the proximal parts of the spiral arteries is often hypertrophied.

11. The vascular arrangements found in the period under review do not reveal a marginal sinus in the sense described by Spanner. The probable circulation in the intervillous space is discussed.

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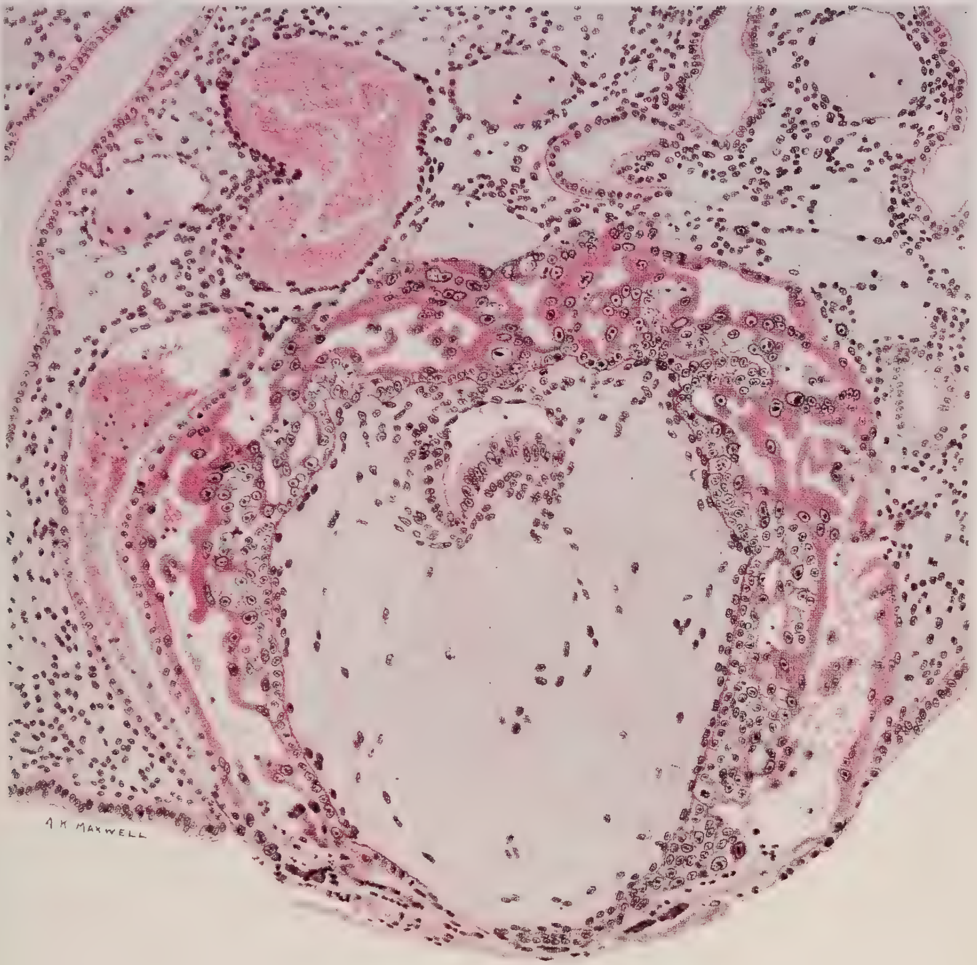
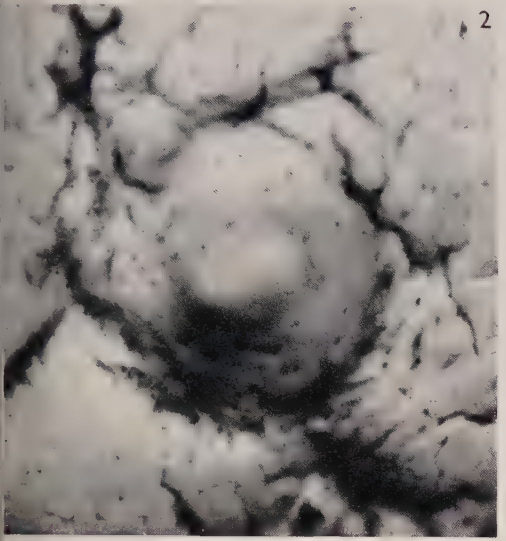
EXPLANATION OF PLATES

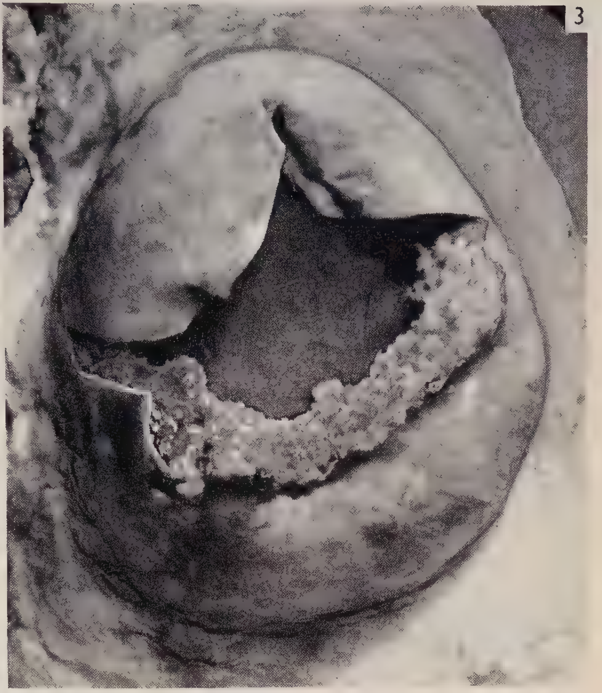
Plate 1

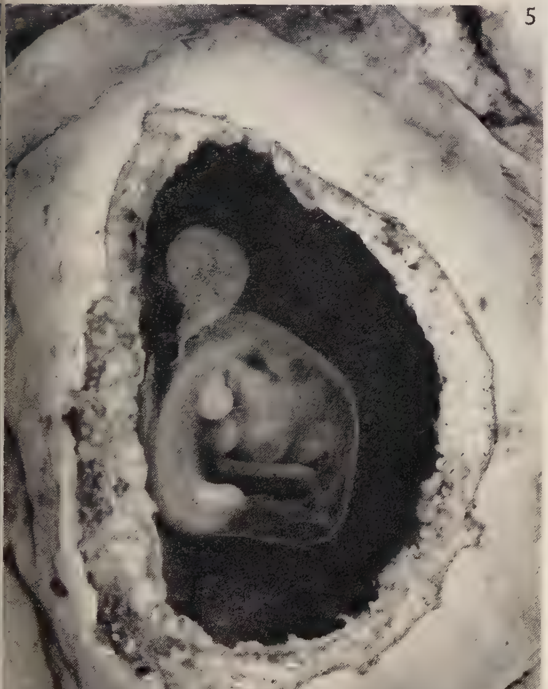
Fig. 1. A drawing (× 140) of a section through the middle of an implantation site at the 11th–12th day of development (Barnes embryo). The surface view of the implantation site is shown in Pl. 2, fig. 2. The blastocystic trophoblast has differentiated into primitive syncytium and cytotrophoblast; the latter surrounds the primitive mesoblast which almost completely fills the original blastocyst cavity. At intervals the cytotrophoblast has proliferated to form projections which are the forerunners of the 'primary villi'. The primitive syncytium, which has not yet extended completely round the superficial aspect of the implantation site, shows an extensive development of intercommunicating lacunae containing some maternal blood cells. The endometrium surrounding the implanted ovum is oedematous and there is haemorrhage into a uterine gland in contact with the syncytium. Photomicrographs of selected portions of the trophoblast of this specimen are illustrated in Pl. 4, figs. 9–12.

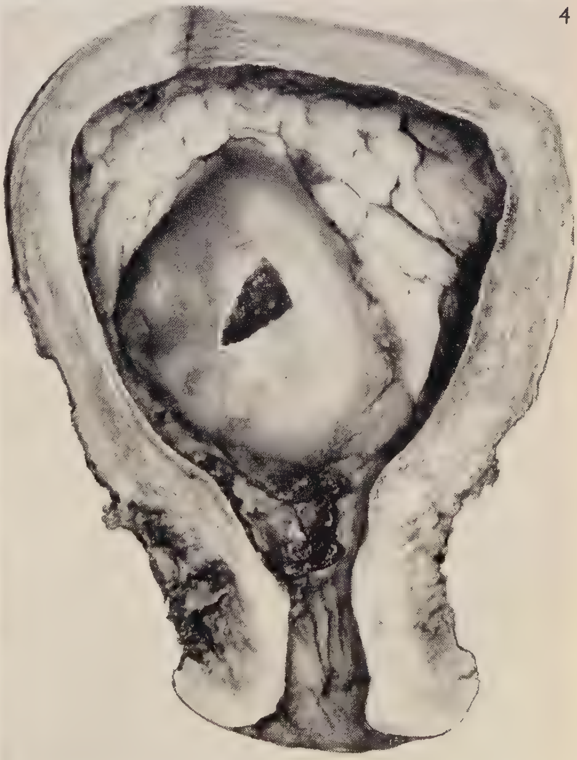


HAMILTON AND BOYD—DEVELOPMENT OF HUMAN PLACENTA

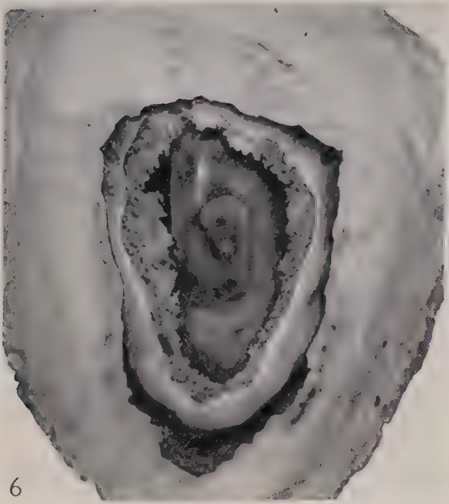








HAMILTON AND BOYD—DEVELOPMENT OF HUMAN PLACENTA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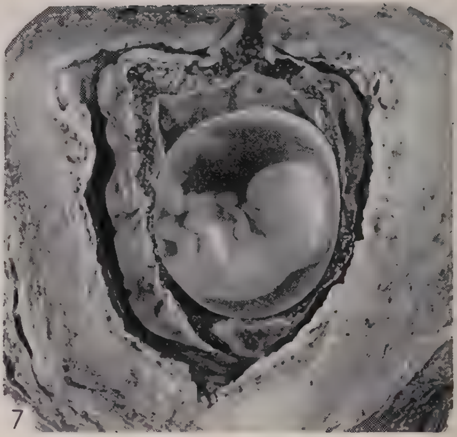
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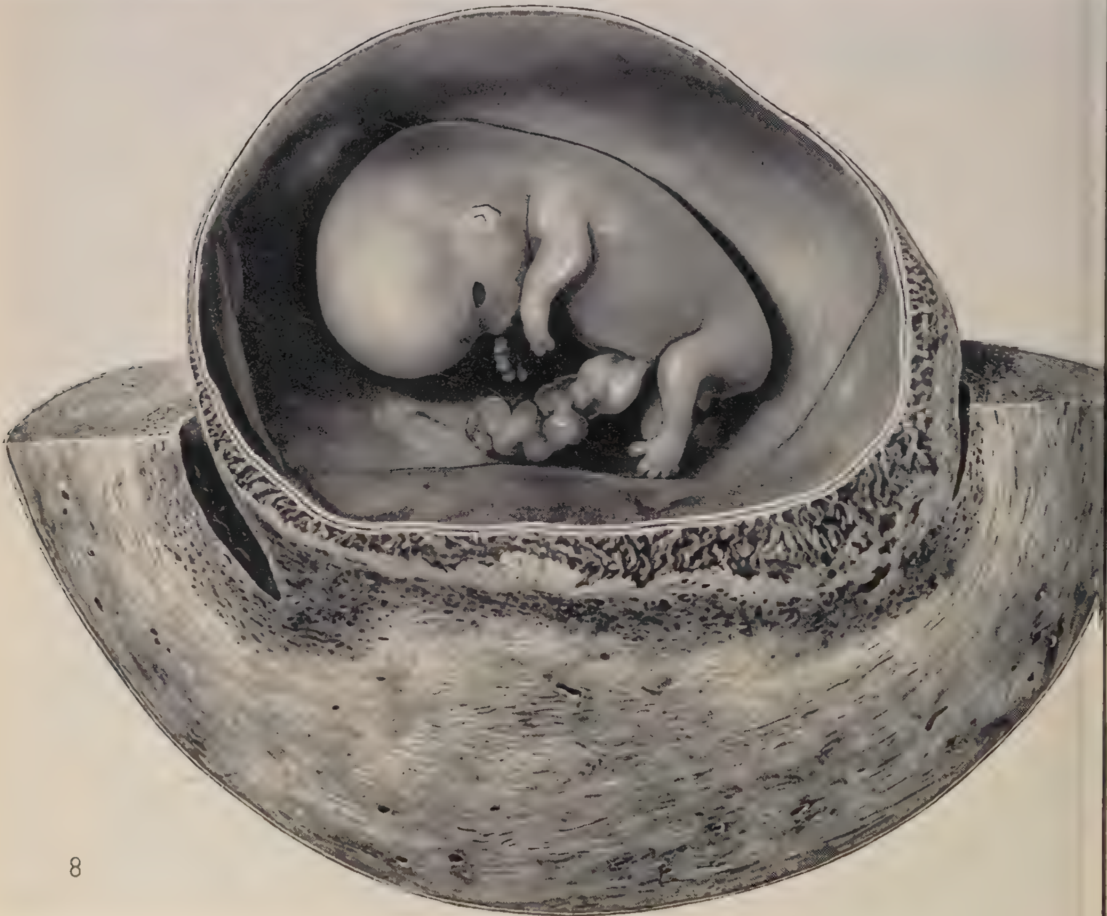
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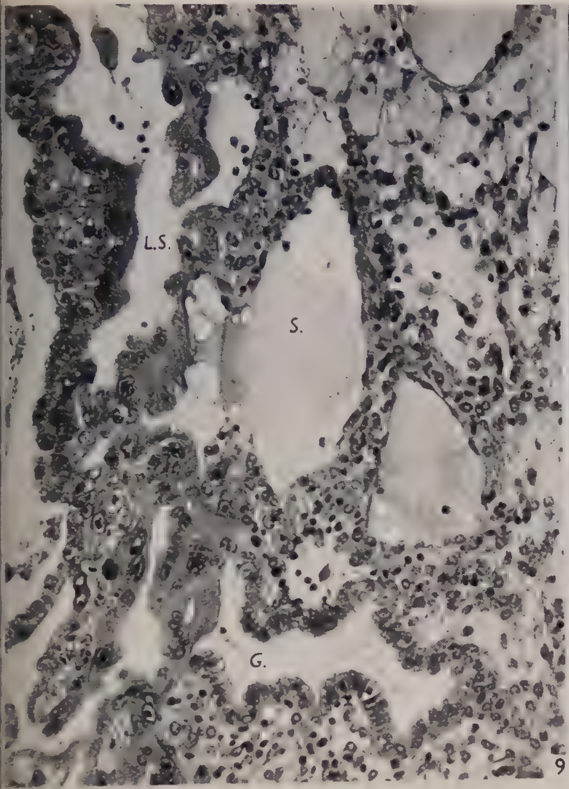

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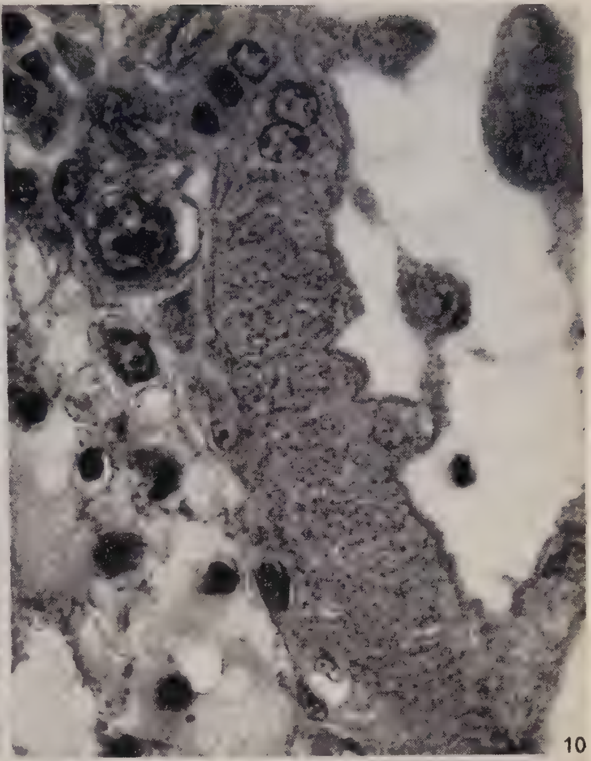

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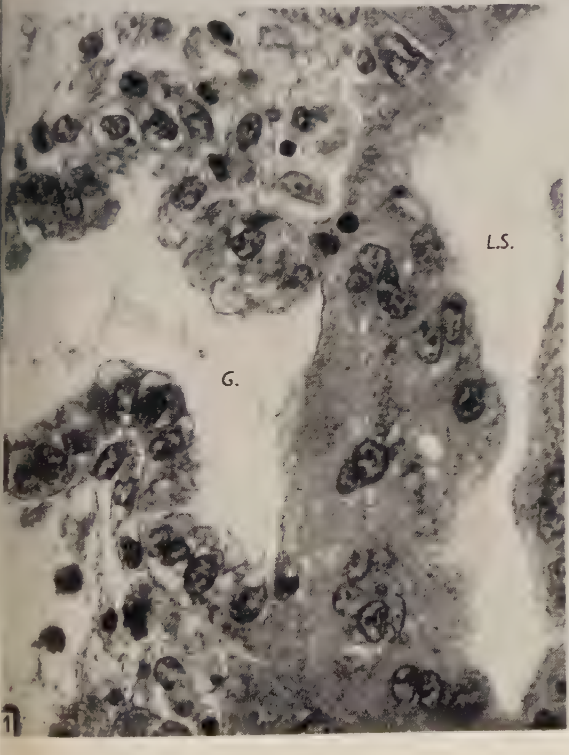

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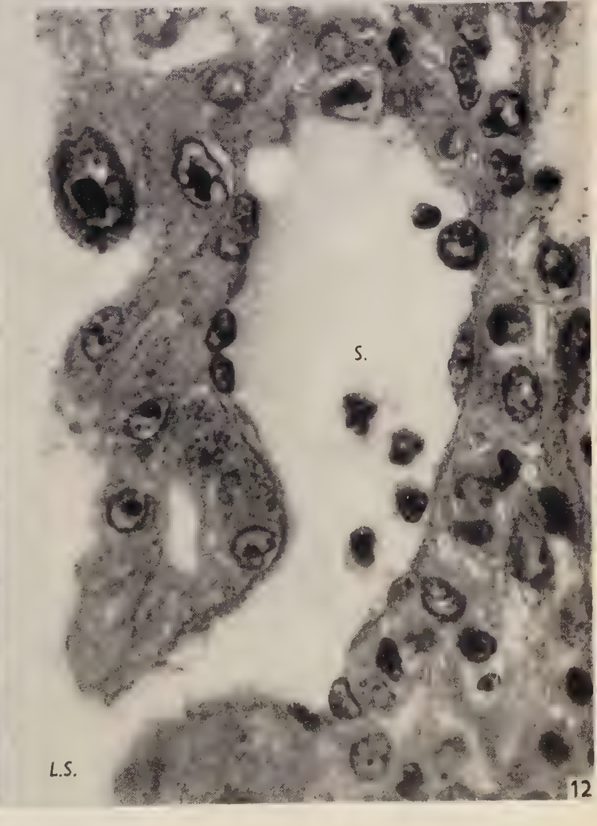

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HAMILTON AND BOYD—DEVELOPMENT OF HUMAN PLACENTA

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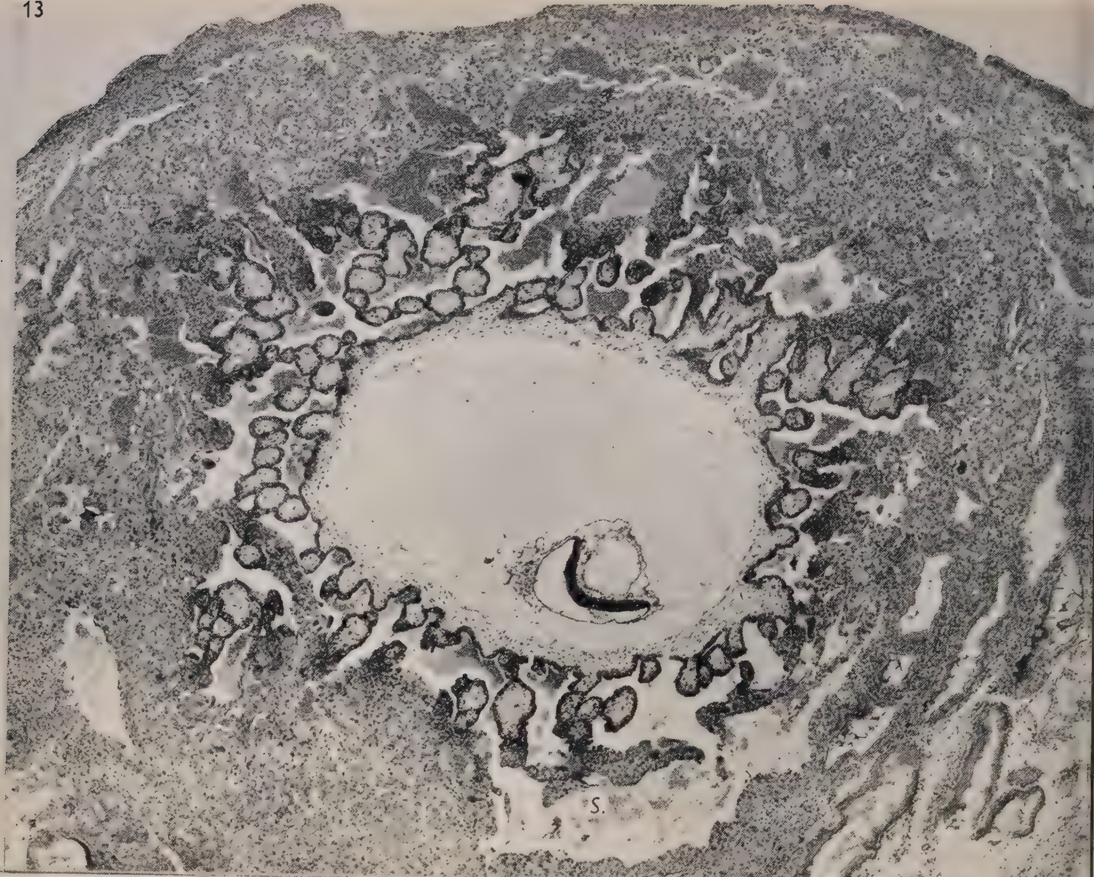

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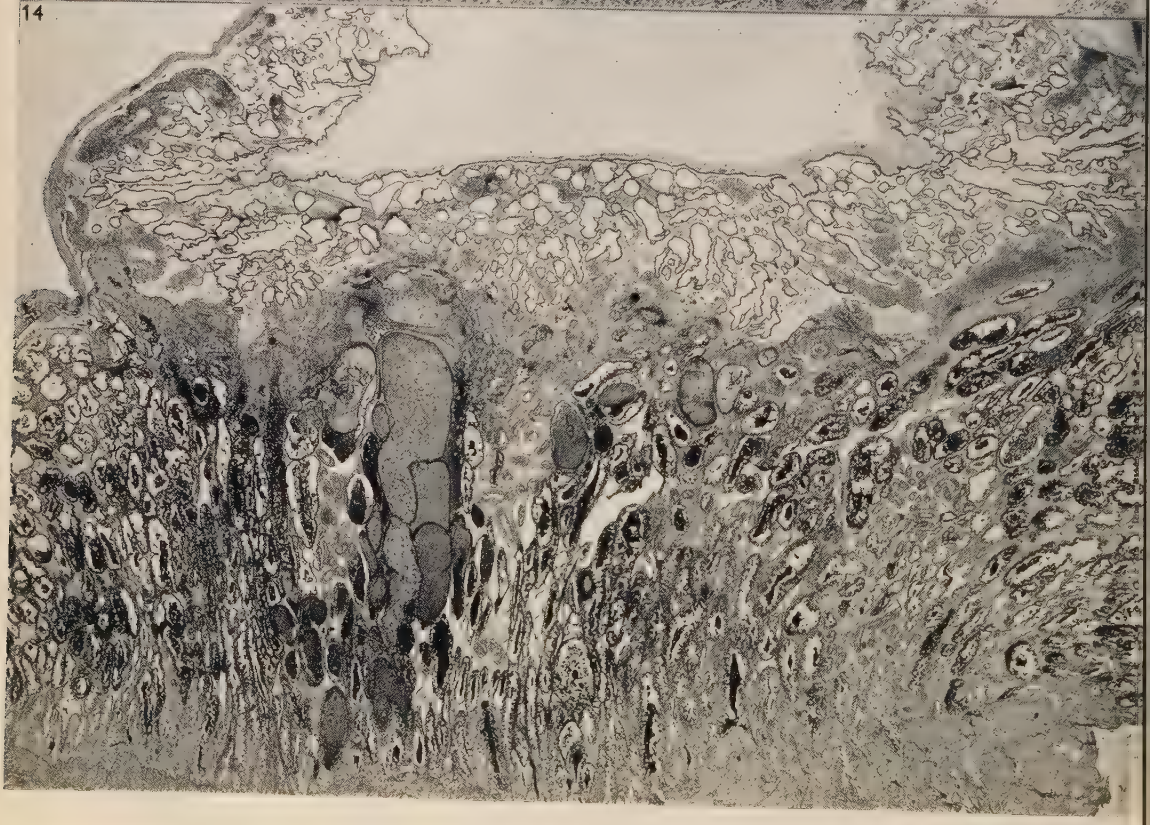

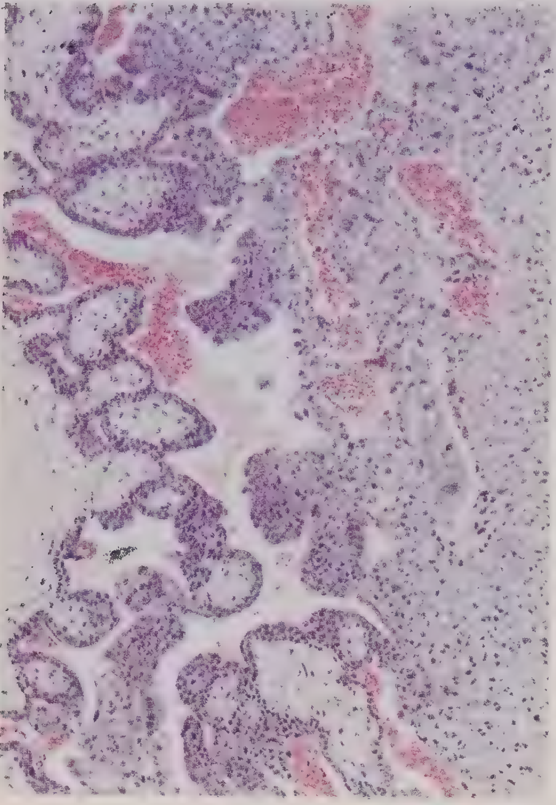

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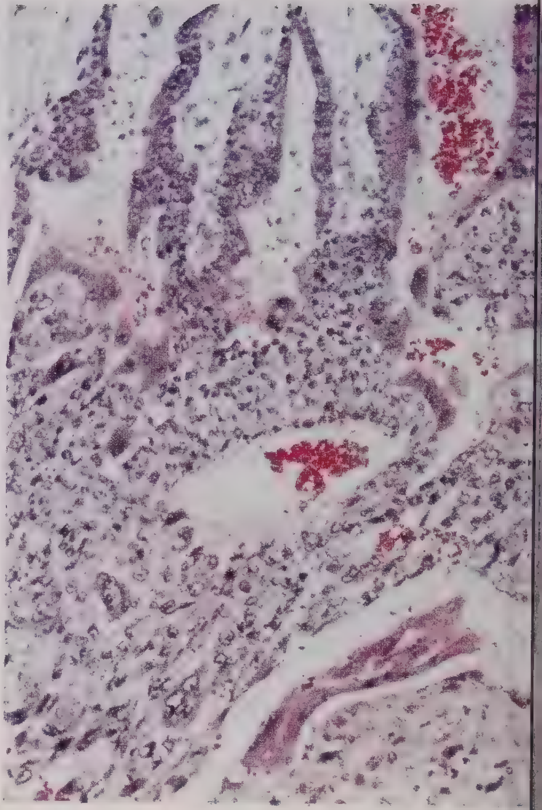

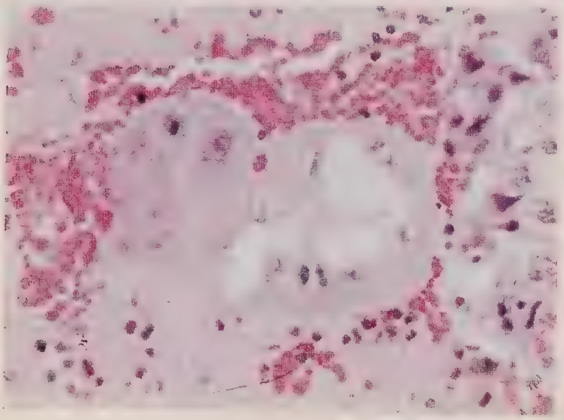

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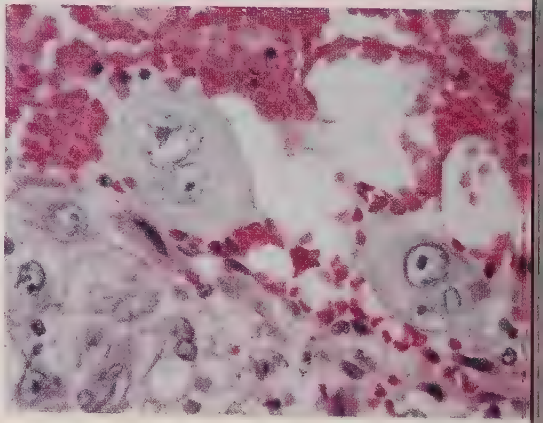

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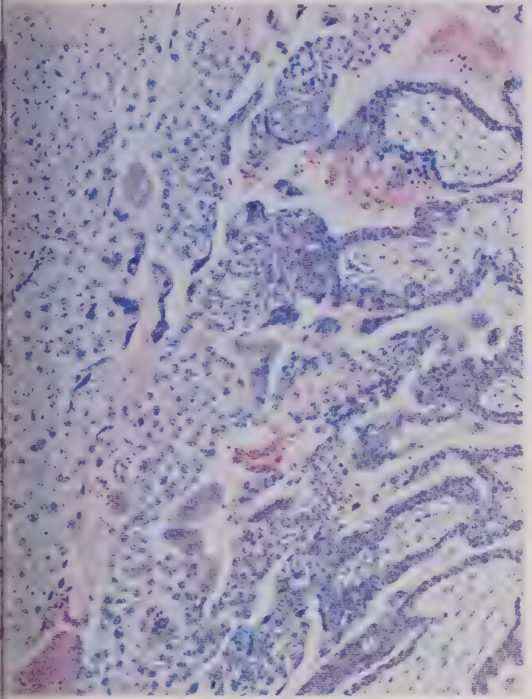

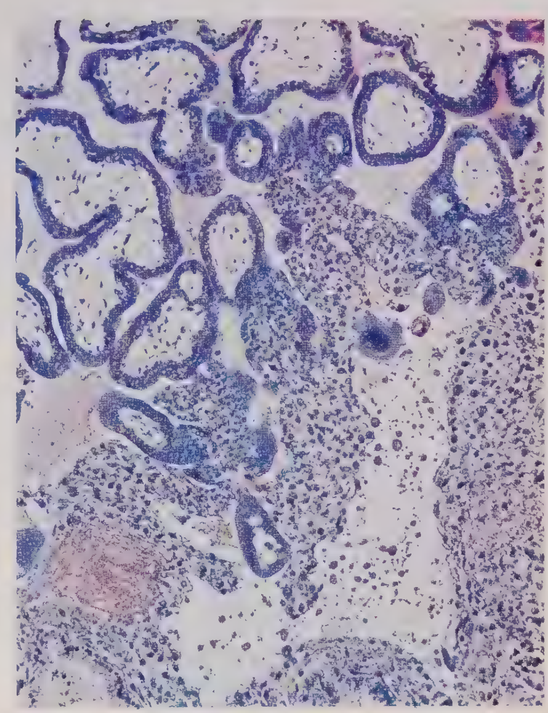

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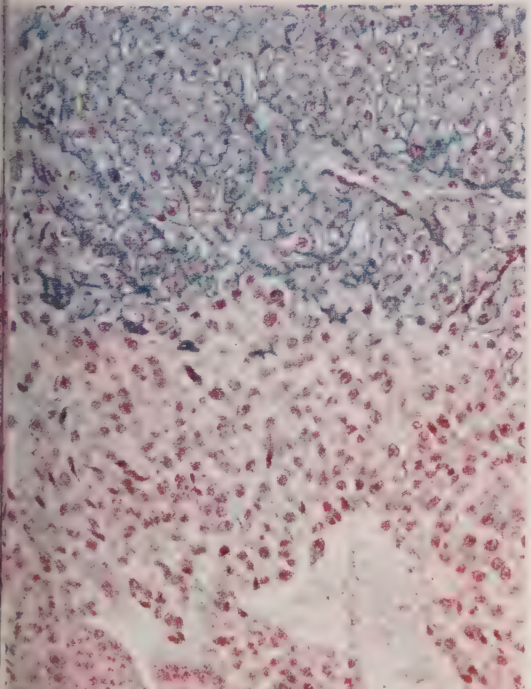

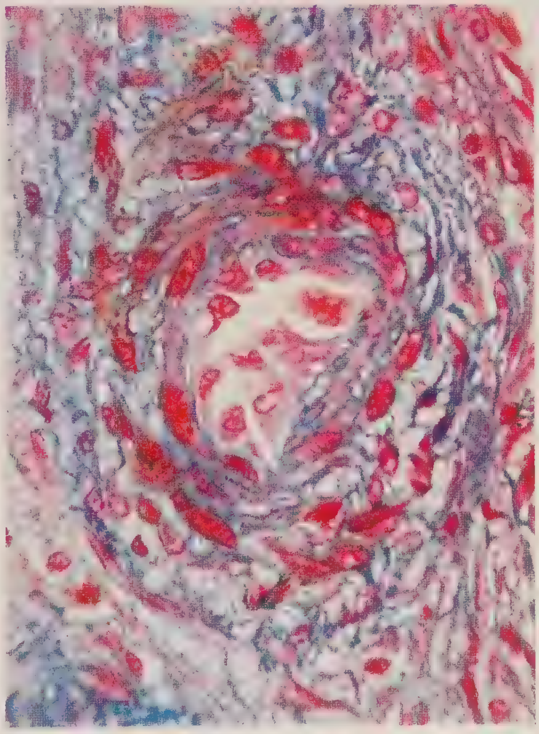

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HAMILTON AND BOYD—DEVELOPMENT OF HUMAN PLACENTA

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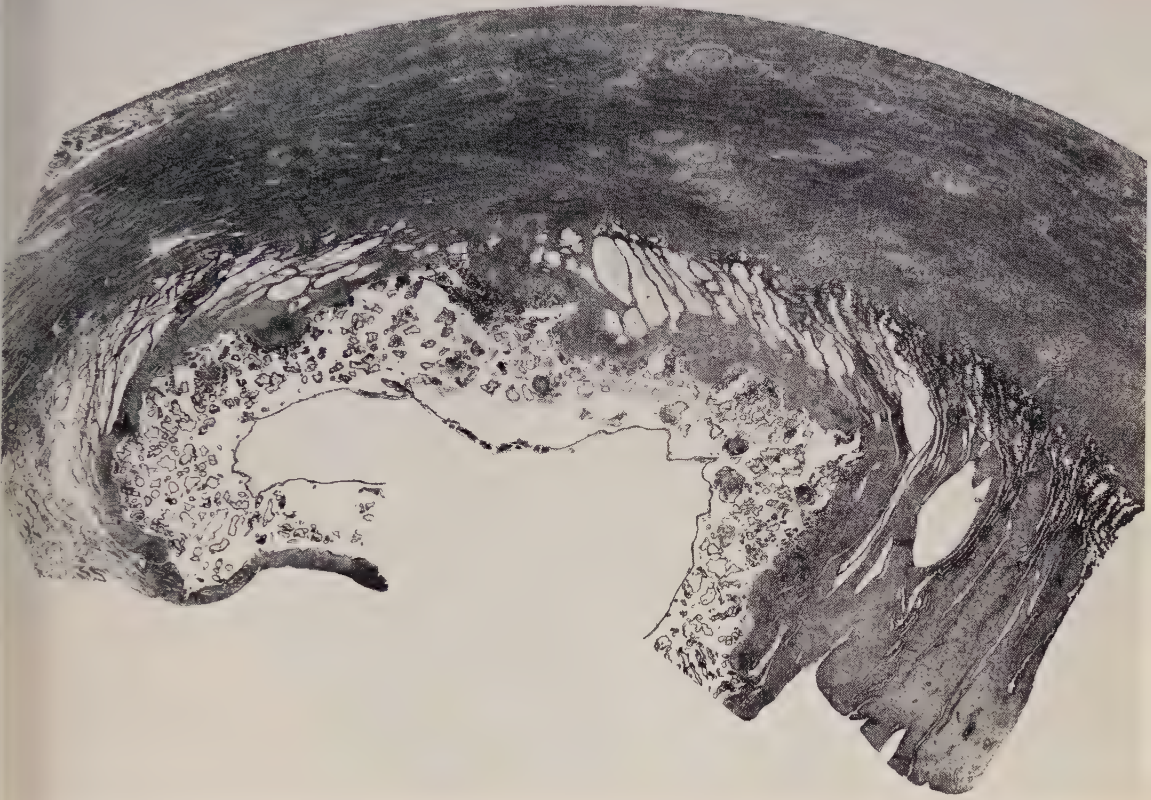

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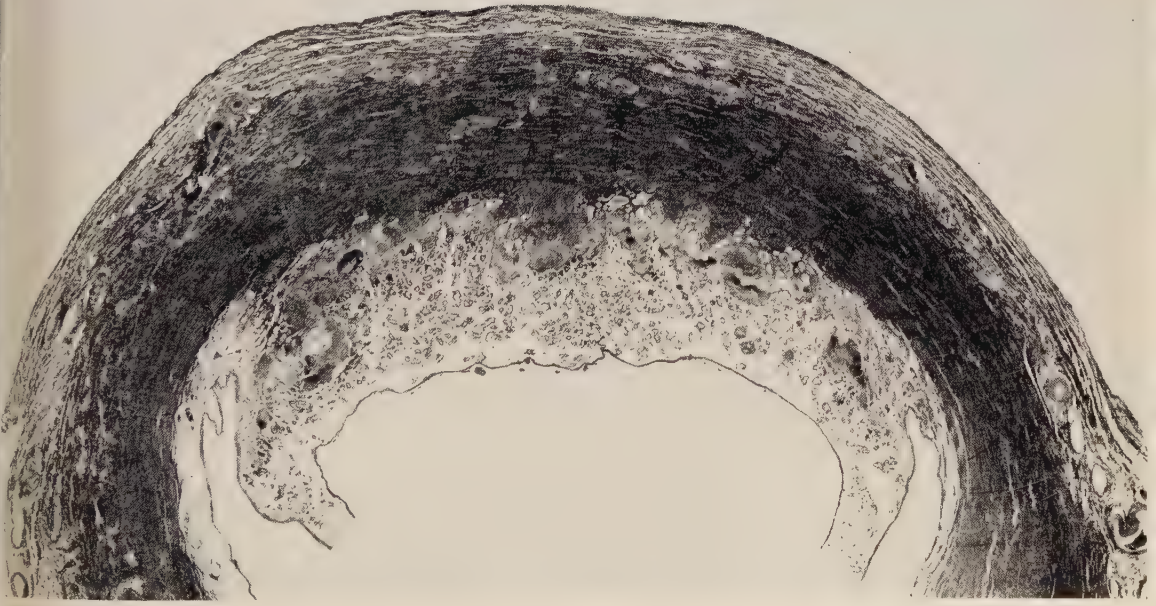

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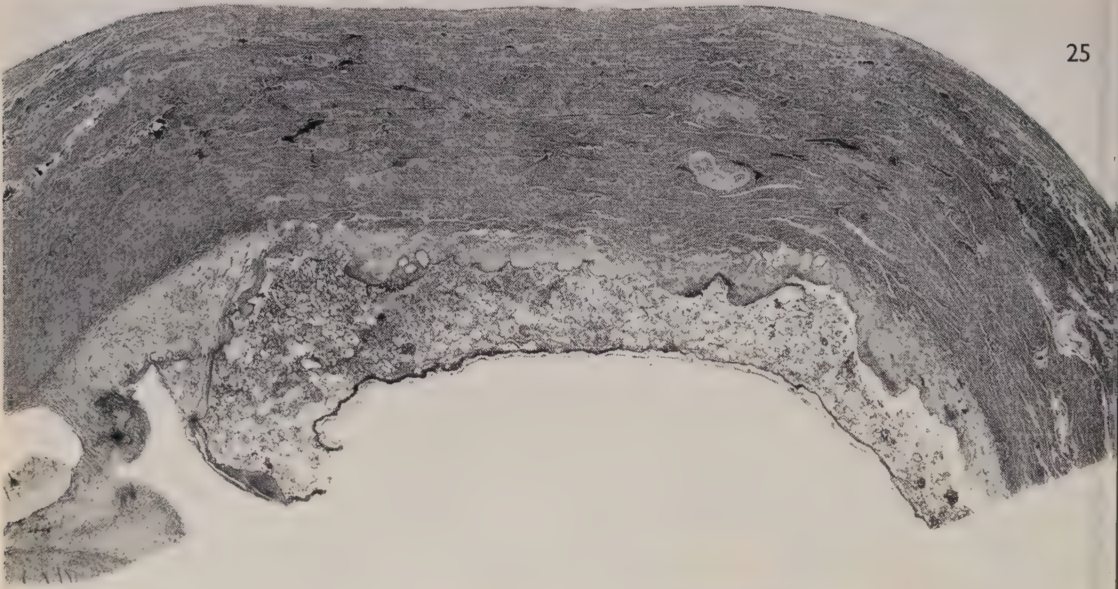

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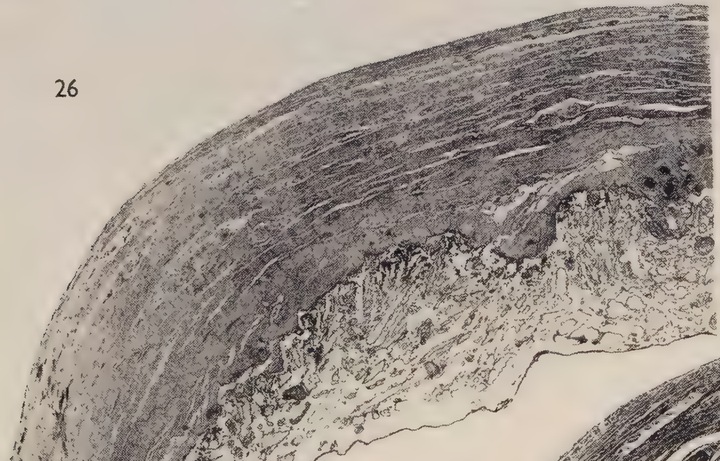

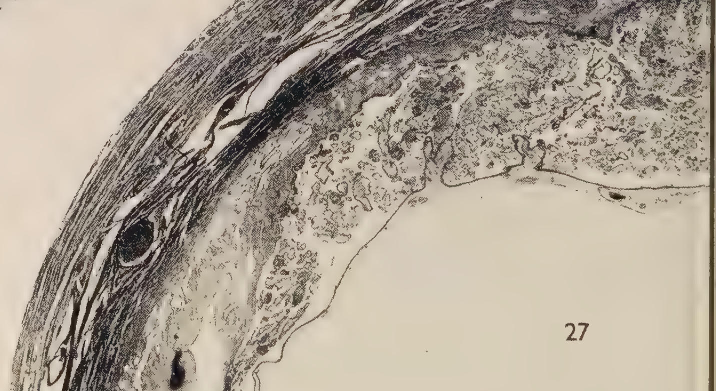

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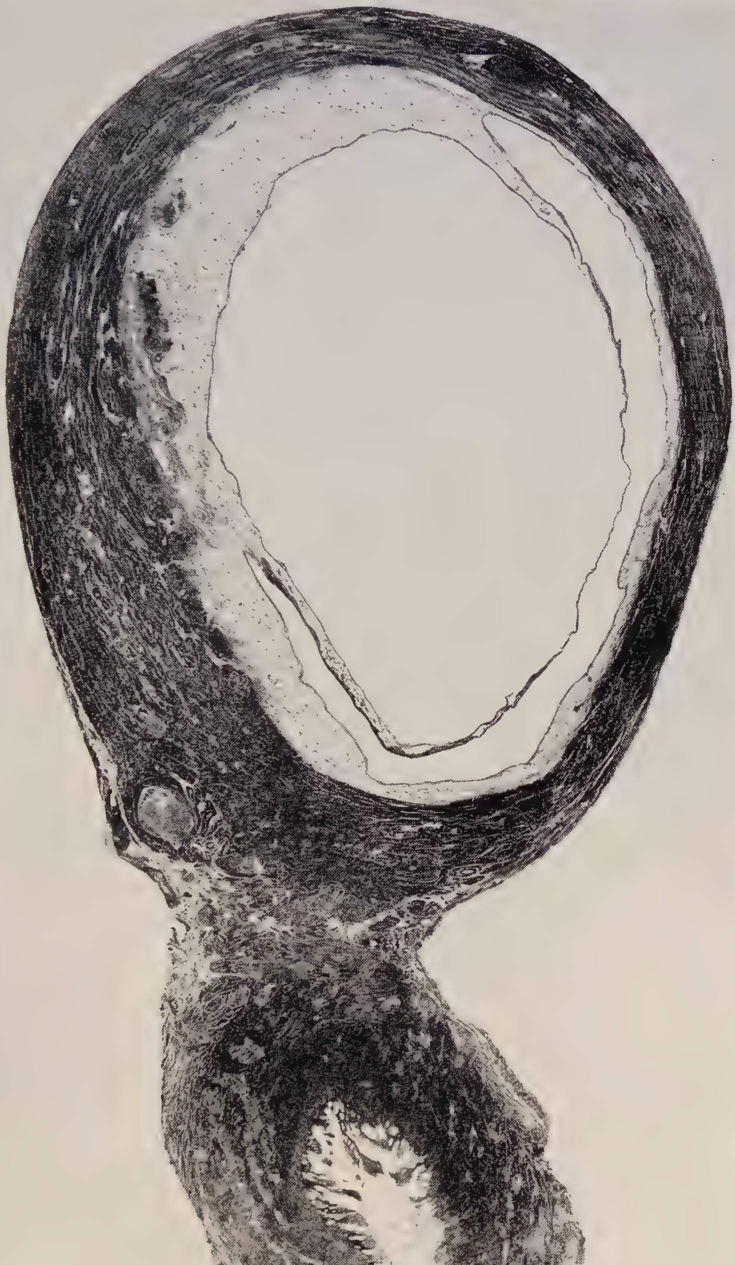

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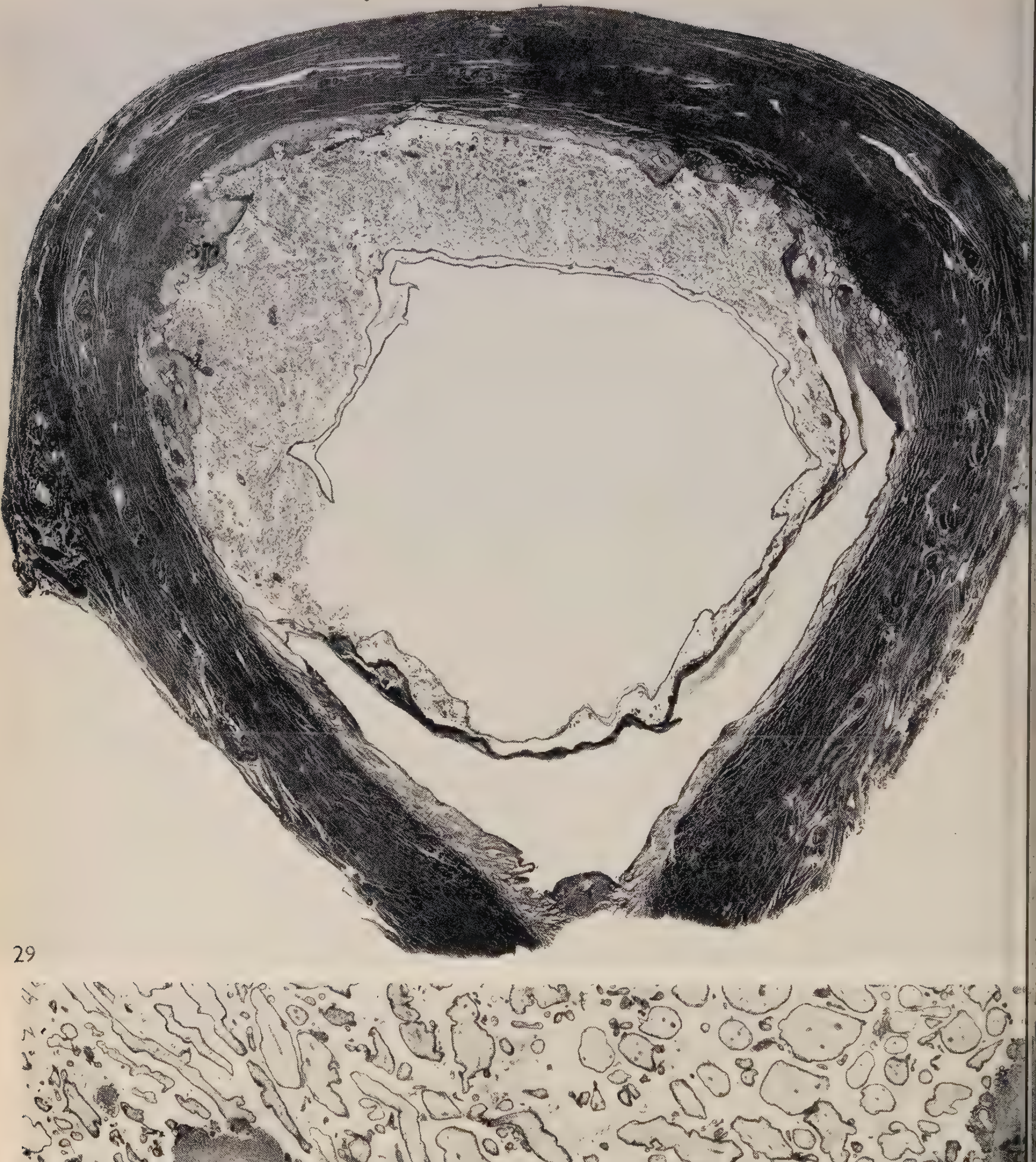

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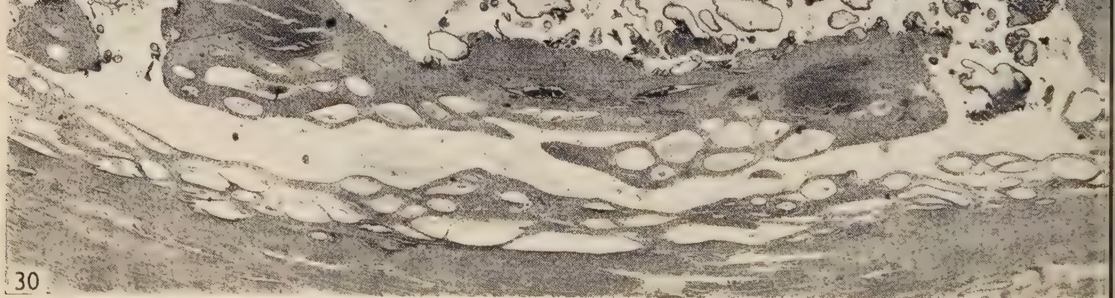

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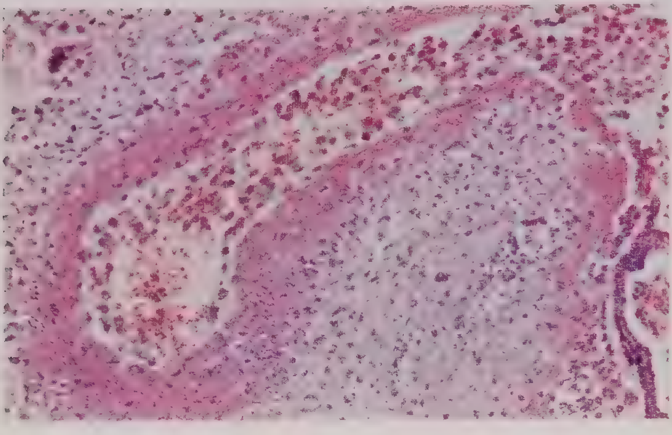

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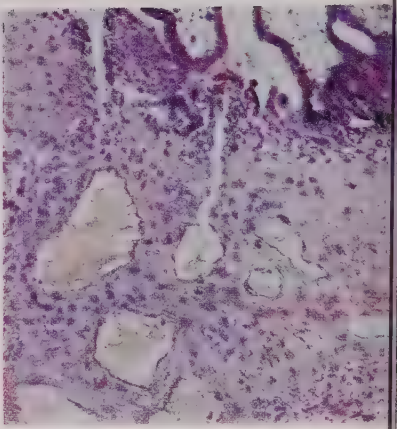

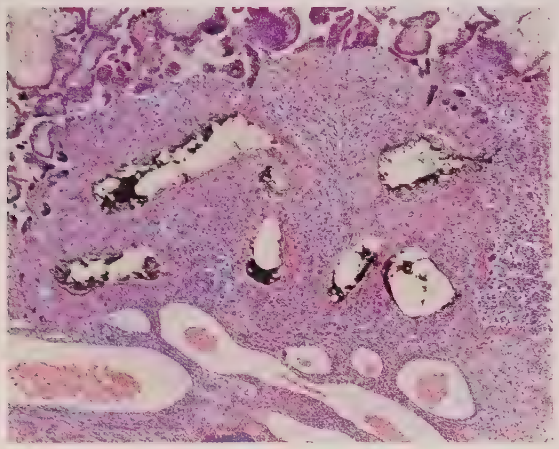

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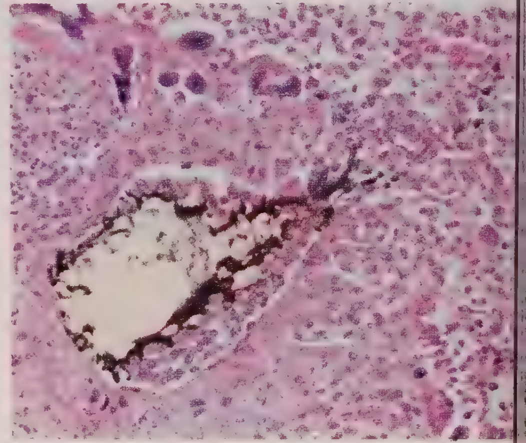

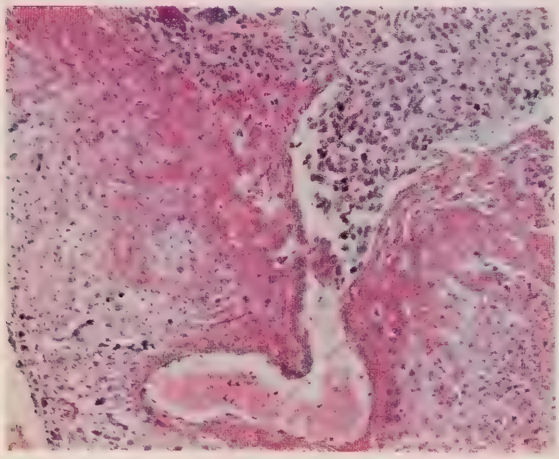

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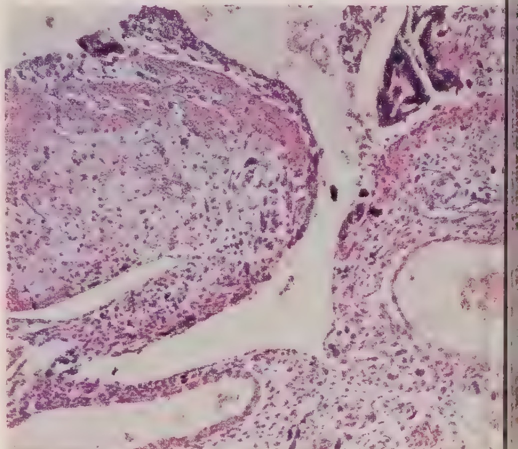

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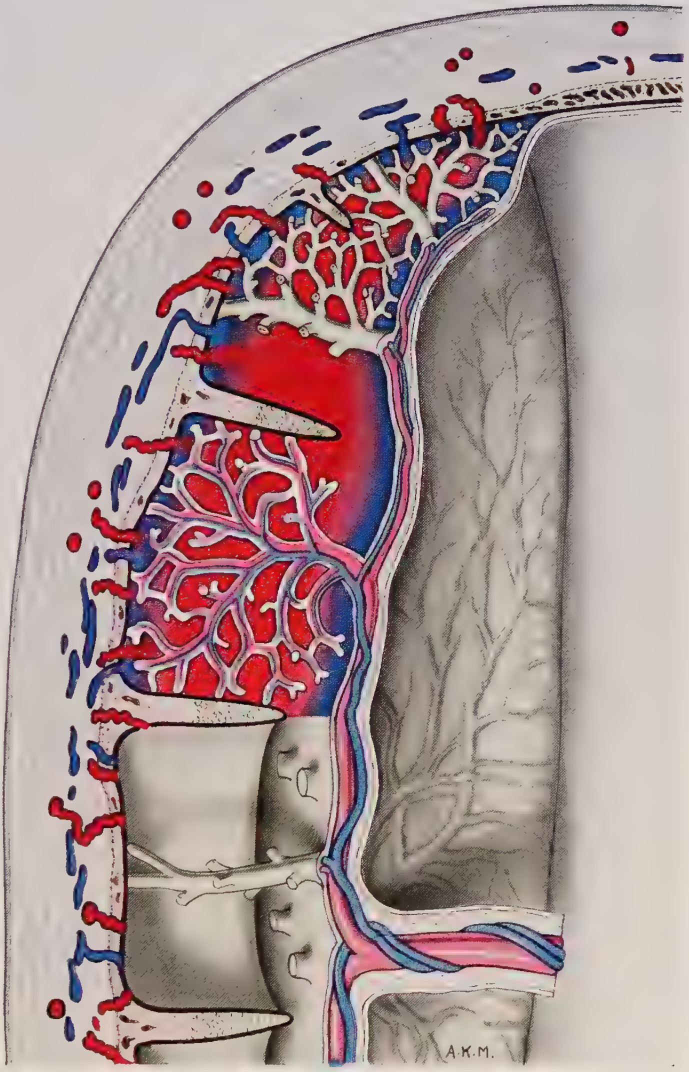

HAMILTON AND BOYD—DEVELOPMENT OF HUMAN PLACENTA

PLATE 2

Fig. 2. Photograph (×12) of the surface view of the implantation site of the Barnes embryo. The implantation site itself appears as a slightly elevated area of the endometrium on which openings of the uterine glands can be seen. The surface of the endometrium shows characteristic shallow and irregular furrows.

Fig. 3. Photograph (×3·5) of the surface view of the partially opened implantation site from which a 28-somite embryo (Camb. H. 710) had been removed. Chorionic villi are seen on the everted cut surface of the decidua capsularis. Photomicrographs of sections through this implantation site are illustrated in Pl. 5, fig. 14 and Pl. 7, figs. 20–22.

Fig. 4. Photograph (×1·1) of a general view of the interior of the uterus containing the implantation site of a 10 mm. embryo (CX. 100). A small opening has been made through the central part of the decidua capsularis. Note the smoothness of this portion of the decidua in comparison with the furrowed decidua vera (parietalis).

Fig. 5. Photograph (×3·5) of the implantation site of a 10 mm. embryo (CX. 100) with the decidua capsularis removed to an extent sufficient to show the embryo, within the collapsed amnion, and the yolk sac. Note the extent of development of the chorionic villi related to the decidua capsularis. This photograph has been rotated in relation to fig. 4 to show the embryo in a suitable orientation. A section through the placenta of this specimen is illustrated in Pl. 8, fig. 23.

PLATE 3

Fig. 6. Photograph (×1) of a coronal section of a uterus containing a 15 mm. embryo (CX. 102). The decidua capsularis has been opened to show the amnion containing the embryo. A section of the placenta of this specimen is illustrated in Pl. 8, fig. 24.

Fig. 7. Photograph (×0·9) of a coronal section of a uterus containing a 29 mm. embryo (CX. 105). Note that the uterine lumen is still widely patent. A section through the uterus and *in situ* placenta of this specimen is illustrated in Pl. 9, fig. 25.

Fig. 8. Photograph (×2) of section through a portion of the uterus to show the placenta and chorionic sac containing a 36 mm. embryo (Camb. H. 789.). The basal placental plate separating the chorionic villi and the intervillous space from the decidua basalis appears as a thin band across the greater part of the attachment area. The decidua basalis, though of variable thickness, is still very distinct. The placental (i.e. lower) half of the decidua capsularis has chorionic villi related to it projecting into an associated extension of the intervillous space. The upper part of the decidua capsularis, together with the abembryonic portion of the chorion, is markedly attenuated. Consequently in this region the interior of the chorionic sac is separated from the uterine lumen by only a very thin double layer of foetal and maternal tissue. The decidua vera can be seen on either side of the placenta extending up to the cut margin of the uterine wall. Note the presence of uterine veins throughout the decidua basalis and the absence of any region of the intervillous space which could be identified as a marginal sinus.

PLATE 4

Fig. 9. Photograph (×200) of a section through the deep central portion of the implantation site of the Barnes embryo; maternal tissue is on the right of the illustration. The intercommunicating lacunar spaces (*L.S.*) are shown surrounded by syncytial trophoblast. Uterine venous sinusoids (*S.*) communicate with these. A plug of syncytium can be seen in direct contact with the lumen of the uterine gland (*G.*).

Fig. 10. Photomicrograph (×840) of a section through the primitive syncytium and adjacent oedematous endometrium in the Barnes embryo; maternal tissue is on the left of the illustration. Note the texture of the syncytial cytoplasm, a long stretch of which is devoid of nuclei. In the upper part of the figure there is an irregular projection of syncytium, containing a very large nucleus, into the endometrium; elsewhere the foetal-maternal junction is smooth.

Fig. 11. Photomicrograph (×636) of the foetal-maternal junction of the Barnes embryo. A mass of primitive syncytium is in direct contact with the lumen of a uterine gland (*G.*) in which secretion can be seen. The syncytium separates this lumen from a lacunar space (*L.S.*).

Fig. 12. Photomicrograph (×840) to show an opening of a venous sinusoid (*S.*) into a lacunar space (*L.S.*). Note the relations of the endothelium of the sinusoid to the syncytium.

PLATE 5

Fig. 13. Photomicrograph (× 45) of a section of the implantation site of a 14-day embryo (Missen). Chorionic villi with mesodermal cores are now well established round the whole of the chorionic sac. There is an extensive intervillous space which, in this section, communicates at two points with a large venous sinusoid (*S.*). Details of the differentiation of the trophoblast in this specimen are shown in Pl. 6, fig. 15.

Fig. 14. Photomicrograph (× 10) of a section of the implantation site of a 28-somite human embryo (Camb. H. 710). Villi which cover the whole of the chorionic sac are well established, and possess numerous foetal vessels. There is no indication of a marginal sinus in the intervillous space; there is, however, venous drainage from the left margin of this space. The decidua basalis is thick and contains many dilated glands; several of the central ones show haemorrhage into their lumina. The effect of expansion of the chorionic sac on the uterine glands is particularly well shown on the right of the illustration where their necks are constricted and stretched, thus damming back the products of secretion. Veins and spiral arteries can be identified at intervals in the decidua basalis.

PLATE 6

Fig. 15. Photomicrograph (× 100) of part of a section (H. & E.) through the foetal-maternal junction of a 14-day embryo (Missen). The chorionic villi (to the left of the illustration) possess mesodermal cores but as yet no definitive blood vessels are present. The villi possess a covering of definitive syncytium overlying Langhan's layer. At the tips of some of the villi cytotrophoblastic columns, by proliferation, are contributing to the trophoblastic shell. On the maternal side of this shell a number of persisting masses of primitive syncytium can be identified. In the intervillous space there are maternal blood vessels and at about the centre of the figure an isolated syncytial sprout in the intervillous space; maternal blood cells are also present in the intervillous space.

Fig. 16. Photomicrograph (× 150) of part of a section (H. & E.) through the tips of the chorionic villi (above) and of the adjacent endometrium (below) in a 16-day human embryo. Cytotrophoblastic columns at the tips of the two central villi have proliferated and fused to form part of the trophoblastic shell. Definitive syncytium can be identified on the surface of the villi and primitive syncytium is related to the shell. Maternal blood vessels and a syncytial sprout can be identified in the intervillous space.

Fig. 17. Photomicrograph (× 200) of a section (H. & E.) through the peripheral syncytium of an 18-day embryo (Gar). The syncytium shows extensive vacuolation and a maternal red blood corpuscle is present in one of the vacuoles. At another point several such red blood corpuscles are in the process of coming to be included within the syncytium.

Fig. 18. Photomicrograph (× 270) of a section (H. & E.) through a uterine gland near the implantation site of an 18-day embryo (Gar). The lumen of the gland possesses maternal red blood corpuscles and two masses of multinucleated syncytium.

PLATE 7

Fig. 19. Photomicrograph (× 100) of a section (H. & E.) of an 18-day embryo (Gar). Marked proliferation at the tips of the villi (to the right) has resulted in the production of a trophoblastic shell which now extends completely around the foetal tissue. The villi are covered except at their tips by a Langhan's layer of cytotrophoblast and a superficial layer of definitive syncytium. At one point a syncytial sprout can be seen arising from the definitive syncytium. Remnants of the primitive syncytium lie within and on the maternal aspect of the trophoblastic shell. Early vasculogenesis is present in the mesodermal cores of the villi.

Fig. 20. Photomicrograph (× 60) of a section (H. & E.) through the tips of the chorionic villi and trophoblastic shell of a 26-day human implantation site (Camb. H. 710). The portion of the trophoblastic shell illustrated contains a typical irregularly shaped space in which glandular secretion and cellular detritus can be identified. There are several masses of primitive syncytium related to the cytotrophoblast. One of these shows extensive vacuolation and an inclusion within one of the vacuoles.

Fig. 21. Photomicrograph (× 150) of a section (Masson's trichrome stain) of the foetal-maternal junction of a 26-day implantation site (Camb. H. 710). The foetal tissue (below) shows the

trophoblastic shell, the tip of a villus with a cytotrophoblastic column and some residual syncytium. Interspersed amongst the typical cytotrophoblastic cells of the shell are darkly staining fusiform cells. In the upper portion of the figure the decidua is surrounded by blue-staining collagen in striking contrast to the appearance presented by the cytotrophoblast.

Fig. 22. Photomicrograph (×350) of a section (Azan stain) through an endometrial spiral artery close to the implantation site of a 26-day embryo (Camb. H. 710). The cells of the endothelium of the vessel are markedly hypertrophied.

PLATE 8

Fig. 23. Photomicrograph (×6) of a section through the uterus and *in situ* placenta of a 10 mm. embryo (CX. 100). The villi are more or less equivalently developed round the whole of the chorionic sac. The decidua capsularis is still relatively thick. The distortion and dilatation of the glands in the decidua basalis, which has been irregularly penetrated by the trophoblast, are clearly shown. The thick decidua vera is well shown on the right of the illustration.

Fig. 24. Photomicrograph (×6) of a section through the uterus and *in situ* placenta of a 15 mm. embryo (CX. 102). The chorionic villi are now distinctly better developed in relation to the decidua basalis and the decidua capsularis is much attenuated. The uterine glands are less apparent than in the specimen illustrated in fig. 23. The very irregular decidua basalis and the decidua vera are both much thinned.

PLATE 9

Fig. 25. Photomicrograph (×2·5) of a section through the uterus and *in situ* placenta of a 29 mm. embryo (CX. 105). The foetal-maternal junction is now very distinct and the decidua basalis is thin. The implantation in this specimen was low on the posterior uterine wall and the cervix can be seen to the left of the illustration. On the right there has been some separation of the chorionic villi from the decidua.

Fig. 26. Photomicrograph (×3·5) of a section through rather less than half of the *in situ* placenta of a 42 mm. foetus (CX. 110) and the related uterine wall.

Fig. 27. Photomicrograph (×4) of a section through rather less than half of the *in situ* placenta of a 50 mm. foetus (CX. 111) and the related uterine wall.

PLATE 10

Fig. 28. Photograph (×1·8) of a uterus which contained a 30 mm. embryo (CX. 106). The decidua capsularis, which is very thin, approaches but does not make contact with the decidua vera. Note the complete absence of any indication of a marginal sinus in the intervillous space.

PLATE 11

Fig. 29. Photograph (×1·8) of a uterus which contained a 60 mm. foetus (CX. 112). The uterine lumen is still apparent in the lower half of the uterus. The decidua basalis is very thin. Distorted and dilated uterine glands are present in the decidua vera to the right of the area of placental attachment.

Fig. 30. Photomicrograph (×18) of a section through the central portion of the *in situ* placenta of a 30 mm. embryo (CX. 106). Two large openings from the intervillous space into uterine veins are shown. A plug of villi can be seen in the right of these communications.

PLATE 12

Fig. 31. Photomicrograph (×100) of a section (H. & E.) of the termination of an endometrial spiral artery in the *in situ* placenta of a 15 mm. embryo (CX. 102). Cytotrophoblast cells are invading and plugging the lumen of the vessel. The wall of the artery shows marked degenerative changes. A portion of syncytium can be seen to the right of the illustration.

Fig. 32. Photomicrograph (×100) of a section (H. & E.) through the foetal-maternal junction in the *in situ* placenta of a 22 mm. embryo (CX. 103). The terminal coils of an endometrial spiral artery can be seen. A straight channel from the lumen of one of the coils extends almost to the intervillous space. Apices of villi and the cytotrophoblastic shell are seen in the upper portion of the illustration.

Fig. 33. Photomicrograph (×110) of a section (H. & E.) through the terminal coils of an endometrial spiral artery and adjacent intervillous space of a 46 mm. embryo (Camb. H. 653). Before fixation a uterine artery in this specimen was injected with indian ink. The ink can be seen in the lumen of the spiral artery and some of it is percolating amongst the cytotrophoblastic cells.

Fig. 34. Photomicrograph (×340) of a section through the termination of the spiral artery shown in fig. 33. The percolation of ink between the cytotrophoblastic cells is clearly seen. Note the highly modified arterial wall and the cytotrophoblastic cells within the arterial lumen.

Fig. 35. Photomicrograph (×70) of a section through the termination of an endometrial artery in the *in situ* placenta of a 50 mm. embryo (CX. 111). The arterial wall shows marked degeneration and cytotrophoblastic cells are extending into the vessel. At each side of the arterial opening Nitabuch's membrane can be seen.

Fig. 36. Photomicrograph (×85) of a section of the central region of an *in situ* placenta of a 50 mm. embryo (CX. 111) showing communication between the intervillous space and a maternal vein. Note the continuity between cytotrophoblastic cells and unaltered venous endothelium. There is no cytotrophoblast within the lumen of the vein but a sectioned syncytial sprout can be seen.

PLATE 13

Fig. 37. Scheme to show the essential features in placental structure which are found after the 60 mm. stage. Three cotyledons, including a marginal one, are illustrated; the cotyledons are separated from each other on the maternal side by the septa. They each contain the group of villi which constitute the associated 'foetal' cotyledon. The villi branch freely and there are many adhesions between adjacent ones so giving a partially labyrinthine nature to the intervillous space. Such villi are shown in relief in the marginal cotyledon and in section in the adjacent one. The third cotyledon has been dissected to show the arrangement of the septa. The openings of the endometrial arteries and veins into the intervillous space through the basal plate are indicated and an attempt has been made to show the probable degree of oxygenation of the maternal blood in the intervillous space. A marginal sinus in the intervillous space has not been included in the scheme as our material does not show such a feature.

COLLAGEN FIBRE PATTERNS IN MAMMALIAN BONE

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From its first appearance in the skeleton of the embryo until death occurs, mammalian bone is subject to two developmental processes. First there are those mechanisms taking place in relation to the periosteum, the endosteum and the epiphyseal cartilages by which the bone grows in length and girth. The greater part of this development is completed by the time the epiphyseal plates are ossified in late adolescence or early adult life, but, even after this time, minor intermittent changes in girth are frequently produced by reactivation of the periosteal and endosteal mechanisms. Associated with this growth process, but not necessarily contributing to it, is a continual change in the internal structure of the tissue. In this process of internal development, bone passes through a series of stages, each of which is characterized by a specific arrangement of the intra-osseous collagen fibres, both in relation to one another and in relation to the vascular spaces of the tissue. It is these differences in the pattern of the collagenous fibres that allows bone to be classified into a number of clearly defined types. The standard classification is that of Weidenreich (1930) which depends largely on the size of the collagen bundles and on the relationship which they bear to neighbouring bundles. In this investigation, however, a somewhat different classification has been found to be advantageous. This is based on three factors, namely, the relationship of neighbouring fibres to one another, their relationship to vascular channels, and the place occupied by the tissue in the developmental history of the skeleton as a whole. The types of bone recognized in this classification are enumerated below in the chronological order in which they appear in the development of mammalian bone: (1) woven-fibred bone; (2) primary osteones; (3) surface bone; (4) secondary osteones (Haversian systems); (5) interstitial bone.

The fibre patterns of secondary osteones, and of interstitial bone derived from secondary osteones, have been considered in a previous publication (Smith, 1959). The present paper is concerned mainly with the other types of bone.

METHODS

The investigation was carried out on the human tibia and femur and on long bones of the ox, horse, pig, sheep, dog and cat. Serial 5 μ paraffin sections were prepared in longitudinal and transverse planes and the majority of these were stained by Weidenreich's modification of Weigert's fibrin stain (Weidenreich, 1923). The interpretation of bone sections stained by this method has been discussed in a previous paper (Smith, 1959) in which it was demonstrated that bundles of collagen fibres running approximately parallel to the plane of section appeared as deep violet lines, whereas those running approximately at right angles to the plane of section appeared as circumscribed pink areas stippled by small violet spots (Pl. 3, fig. 1). The violet spots were interpreted as the fibres comprising the bundle and the enveloping pink area as an interfibrillar substance. In addition, ground transverse sections of whole bones were prepared and examined, after decalcification and clearing, by polarized light. This method was found useful for assessing the gross structure of large areas of bone but it was not used in the study of its fine structure.

OBSERVATIONS

Woven-fibred bone

Woven-fibred bone consists of a calcified matrix which is permeated by collagen fibres, and throughout it there are lacunae containing osteocytes. When it is first laid down it contains numerous large vascular spaces, but as its development proceeds these spaces become occupied by primary osteones.

The collagen fibres in woven-fibred bone are of two kinds which differ in appearance, and development. Thus the calcified matrix is always permeated by a meshwork of collagen bundles which bear no fixed relationship either to the lacunae or to the vascular spaces of the tissue. These bundles do not lie parallel to one another as is the tendency in all other types of bone; nevertheless, they usually show some degree of preferential orientation in a direction parallel to the long axis of the bone. In longitudinal sections (Pl. 1, fig. 1) the fibre bundles are therefore usually seen as interweaving fibrillar bands which stain violet purple in colour, whereas in transverse section (Pl. 1, fig. 2) they appear as polygonal areas which stain pink and are stippled by violet spots. The diameter of these bundles is not appreciably greater than that of the bundles found in other types of bone (Pl. 3, fig. 1), and it is considered that the alternative designations of *coarsely bundled bone* and *coarse-fibred bone* are unsuitable.

This type of collagen bundle is a constant feature of woven-fibred bone and the contained fibres will be referred to as the intrinsic fibres of the tissue. In many areas of woven-fibred bone, though not in all, other collagenous fibre bundles are also present. These are the elements commonly known as Sharpey fibres or perforating fibres. They are considerably larger than the intrinsic fibre bundles described above, a diameter of 10–12 μ being common, and they stain a deeper violet with the Weidenreich method. In longitudinal section (Pl. 1, fig. 3) the bundle consists of a variable number of fine, dark violet fibres interweaving with one another, whereas in transverse section (Pl. 1, fig. 4) they are seen as large pink areas stippled with deep violet spots. In many sections the bundles appear to have shrunk eccentrically from the surrounding tissue leaving a narrow peri-fibrillar space at one side. It is considered that these, like many spaces of similar appearance, are to be regarded as artefacts.

It is generally accepted that Sharpey fibres represent originally extra-osseous collagen bundles which have been included within the bone during its growth. This view is in conformity with the continuity which often exists between intra-osseous bundles and collagen bundles lying both outside the bone (Pl. 1, fig. 5) and in the vascular spaces within the bone (Pl. 1, fig. 6). Sharpey fibres in bone invariably stain deeply with the Weidenreich method, but their continuations both outside the bone and into its vascular spaces are unstained.

As might be expected from this mode of formation Sharpey fibres are often, though not invariably, arranged in groups running in parallel directions (Pl. 1, fig. 7), the direction varying from one group to another.

The vascular spaces which are included within woven-fibred bone as it is laid down form a continuous labyrinth throughout the tissue. In some regions this labyrinth is irregular and resembles the cavity within a sponge (Pl. 1, fig. 8), but in others the cavities of the labyrinth are preferentially orientated in one or two directions. Thus by examination of serial sections, it can be established that the vascular spaces in the posterior part of the femoral shaft in a 170 mm. human foetus (Pl. 1, fig. 9) are wide cylindrical canals which extend in a longitudinal direction and anastomose infrequently with one another. On the other hand, the vascular spaces in the lateral part of the same bone (Pl. 1, fig. 9) take the form of a series of parallel fenestrated clefts. The clefts extend longitudinally and circumferentially and have infrequent radial connexions with one another. The general form of these clefts can be appreciated from the reconstruction of the primary osteones which subsequently form within them (Pl. 2, fig. 3). Furthermore, it may be inferred from the form of certain primary osteones in the metacarpus of the ox (see below, Pl. 2, fig. 5) that at some stage in the development of that bone, vascular spaces exist in woven-fibred bone as a series of parallel, fenestrated clefts, similar to those described in the human femur, but extending in *radial* and longitudinal directions. Such spaces have not, however, been observed because of the difficulty of obtaining material of the correct age.

Primary osteones

Development and gross form

Primary osteones are invariably formed by the deposition of parallel-fibred bone on the walls of the vascular spaces which lie within woven-fibred bone. Just as those vascular spaces form a continuous labyrinth so the bone laid down within them is a continuum. It is therefore erroneous to regard primary osteones as individual entities or units: they are tracts of bone which have exact limits in the transverse plane but whose extent in a longitudinal direction cannot be exactly defined.

Two of the main features exhibited by primary osteones result from their mode of development. First, it is evident that the gross shape of a primary osteone is dictated by the shape of the vascular space in which it develops. Secondly, it is apparent that as long as it is not eroded by subsequent developmental processes, a primary osteone must always be completely surrounded by woven-fibred bone.

As has been previously noted, the vascular spaces within many regions of woven-fibred bone form a continuous and irregular labyrinth of intercommunicating spaces (Pl. 1, fig. 8). The initial stage in the formation of primary osteones in such a region may be the subdivision of the labyrinth by a number of bridges of pre-osseous tissue (Pl. 1, fig. 10). Parallel-fibred bone is then laid down on the walls of the vascular spaces as an extensive and convoluted layer (Pl. 1, fig. 11, *a*). Subsequently, further bridges of pre-osseous tissue form across the remains of the vascular spaces (Pl. 1, fig. 11, *b*). These bridges extend for some distance in a longitudinal direction dividing the original labyrinth into a variable number of wide longitudinal vascular channels. Thereafter the calibre of each channel is further diminished by the laying down of additional parallel-fibred bone on its walls (Pl. 1, fig. 11, *c*). Thus a fully developed primary osteone of this type is seen in transverse section as an irregular area of parallel-fibred bone embedded in a matrix of woven-fibred bone (Pl. 2, fig. 1). It contains a variable number of longitudinal vascular channels (three in the example illustrated) and these can be traced, in serial transverse sections, for considerable distances, establishing frequent communications with the channels of neighbouring osteones. This change in the vascular pattern from an extensive and irregular labyrinthine cavity in woven-fibred bone, to a series of narrow longitudinal channels in primary osteones is very striking and is associated with a considerable change in the physical properties of the tissue (Smith & Walmsley, 1959).

In those regions in which the vascular pattern of woven-fibred bone is preferentially orientated in one or two directions (see above), the process by which primary osteones are formed is similar to that described above, but their final gross form is different. Thus the primary osteones which are formed within the simple cylindrical cavities in the posterior part of the human femur (Pl. 1, fig. 9) are illustrated in Pl. 2, fig. 2, *a*. Here no division of the original cavities occurs and parallel-fibred bone is laid down in a continuously concentric fashion so that it eventually encloses a single longitudinal vascular channel.

In those cleft-like spaces which are elongated in longitudinal and circumferential directions (Pl. 1, fig. 9) primary osteones develop through the same stages as those forming in an irregular labyrinth. Their definitive shape is illustrated by the wax-plate reconstruction in Pl. 2, fig. 3. They form a series of fenestrated walls, extending longitudinally and circumferentially. The walls are parallel to one another and to the endosteal and periosteal surfaces of the bone, and have infrequent radial connexions. Within the osteones numerous vascular canals (black) run longitudinally and these are connected to one another by occasional horizontal branches. In transverse section such osteones are seen as fusiform areas of parallel-fibred bone which are elongated in a circumferential direction (Pl. 2, fig. 4). They lie in a matrix of woven-fibred bone and contain a variable number of longitudinal vascular channels which are strictly aligned with one another. Lastly, there are those primary osteones which are presumed to develop in vascular clefts which are elongated in longitudinal and radial directions. These have a gross form very similar to that of the type just described, except that in transverse section, they are elongated in a radial rather than a circumferential direction (Pl. 2, fig. 5). It is thus convenient to classify primary osteones, according to their shape and orientation, into *irregular*, *cylindrical*, *circumferential* and *radial* types.

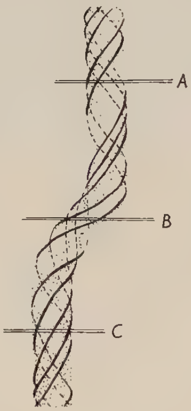
Primary osteones are by definition always formed within vascular spaces which have been included within woven-fibred bone in the process of its formation, and never in cavities created in pre-existing bone by erosion. It is because the formation of primary osteones is thus never associated with a change from a destructive to a formative process that no cement (reversal) lines ever demarcate them from the surrounding woven-fibred bone.

In the previous sections it was noted that the Sharpey fibres which are found in many regions of woven-fibred bone, are often continuous with collagen bundles lying within the intra-osseous vascular spaces (Pl. 1, fig. 6). Where they are present these collagen bundles become included within the primary osteones as the latter are formed, and simultaneously develop a staining reaction identical with that of the Sharpey fibres with which they were originally associated. The outer parts of many primary osteones are therefore penetrated by Sharpey fibres from the surrounding woven-fibred bone (Pl. 2, fig. 8).

The fibre pattern

The fibre pattern in primary osteones varies considerably. The simple cylindrical osteone is usually seen in transverse sections as an annular zone, surrounding a single vascular canal, and composed of pink areas which are stippled with violet spots. These are packed closely together and show no differentiation into lamellae (Pl. 2, fig. 2, *a*). From this appearance it may be inferred that the fibres of the osteone are all arranged parallel to one another in a predominantly longitudinal direction. However, within an area dominated by this type of osteone the appearance illustrated in Pl. 2, fig. 2, *b*, is occasionally seen. Here about two-thirds of the osteone is similar in appearance to the common form, but the rest of the annulus is composed of dark purple fibres running approximately in the plane of the section.

It is considered that this pattern may be explained by certain features which are apparent in longitudinal sections of regions of bone containing similar osteones (Pl. 2, fig. 6). In such a section it is evident that although the vascular canals run for the greater part of their course parallel to the long axis of the bone, they are occasionally kinked; that is they undergo abrupt and temporary deviations. In Pl. 2, fig. 6, four deviations of this kind are indicated by arrows, and it will be noted that whereas the two on the left occur in the plane of the section, those on the right occur at right angles to that plane, so that in each case the osteone surrounding the canal is cut tangentially. Where such a tangential section occurs the collagen fibres are always seen running at an angle of about 20° to the long axis of the vascular canal (Pl. 2, fig. 7) and from this it may be argued that although the collagen fibres of these primary osteones are preferentially orientated in a longitudinal direction, in fact they form steep and continuous spirals, with a helix angle of about 70°, around the vascular canals as illustrated in Text-fig. 1. A transverse section through the strictly longitudinal parts of such a primary osteone (*A* and *C* in Text-fig. 1) would cut all the fibres at the same angle of 70°, giving the appearance shown in Pl. 2, fig. 2, *a*, whereas a transverse section which passed through one of the occasional deviations (*B* in Text-fig. 1) would cut the fibres on one side of the osteone at right angles and those on the other side almost longitudinally giving the appearance shown in Pl. 2, fig 2, *b*.



Text-fig. 1. Spiral arrangement of fibres in a cylindrical primary osteone.

In the larger multi-channelled type of primary osteone (Pl. 2, figs. 1, 4, 5 and 8) a large part of the unit contains distinct lamellae of circumferential and longitudinal collagen fibre bundles. In transverse sections (Pl. 2, fig. 8) the circumferential lamellae are seen as dark fibrillar bands and the longitudinal lamellae as rows of discrete pink areas stippled with dark violet spots. Characteristically the inner lamellae encircle the individual vascular canals, whereas the outer lamellae envelop the whole osteone (Pl. 2, fig. 1).

This lamellated bone is often, though not always, associated with bone of two other fibre patterns. Thus in the most central part of primary osteones, the tissue frequently has an amorphous appearance, in which the collagen fibres cannot be distinguished (Pl. 2, figs. 5, 8). On the other hand, the outermost part of an osteone frequently contains only longitudinal collagen fibres which are not arranged in distinct lamellae (Pl. 2, figs. 2, 4 and 8).

Surface bone

Surface bone forms on the periosteal and endosteal aspects of mammalian diaphyses at various stages during their development. It is most commonly seen as thin layers of parallel-fibred bone clothing the surfaces of a bone shaft which has reached its definitive size (Pl. 4, fig. 3). In some bones, however, either the periosteal or the endosteal surface bone may form a layer of much greater thickness so that it comprises the greater part of the width of the diaphysis (Pl. 4, figs. 2, 7). In others a thin layer of periosteal surface bone may form during growth, and then become included (see below) within the diaphysis by superimposed woven-fibred bone (Pl. 3, figs. 4, 5).

In the most common form of surface bone, the collagen fibres are arranged in alternate lamellae of longitudinal and transverse bundles, running parallel to the surface of the bone (Pl. 2, fig. 9; Pl. 3, fig. 1). In some regions, however (Pl. 3, fig. 2), the longitudinal fibres predominate and the lamellae of transverse fibres are thin and fragmentary and are in places indistinguishable. It is evident that in such regions lamellation of the tissue is much less distinct or entirely absent.

When surface bone is formed in a region not previously ossified, Sharpey fibres, similar in all respects to those previously described in woven-fibred bone, are frequently included within it. In Pl. 3, fig. 3, Sharpey fibres traverse an area of surface bone at right angles to the plane of section, and the shrinkage of the fibres from the surrounding tissue (see above) accounts for its vesiculated appearance. In Pl. 3, fig. 6, on the other hand, Sharpey fibres can be seen traversing surface bone in the plane of the section. The blood vessels in surface bone are included within the tissue during its formation and their pattern varies with the width and position of the layer. In thin layers of either periosteal or endosteal surface bone, the tissue is traversed by only a few radially disposed vessels (Volkmann's canals) (Pl. 4, fig. 1). In a thick layer of endosteal surface bone (Pl. 4, fig. 7) the radial vessels are more numerous. Moreover, they give off longitudinal branches which are constantly aggregated along exact circumferential planes. On the other hand, in a thick layer of periosteal surface bone the radial vessels are less numerous while the longitudinal vessels are arranged in a random fashion (Pl. 3, fig. 3; Pl. 4, fig. 2). In lamellated surface bone the lamellae do not encircle these vascular channels as they do in secondary osteones. On the contrary they simply diverge on either side of them before regaining their original direction (Pl. 3, fig. 3).

Secondary osteones

The development of secondary osteones has been described by Petersen (1930). The first stage in their formation is the creation of tubular cavities within the bone, by the absorption of the tissue surrounding pre-existing vascular canals in primary osteones or in surface bone.

Subsequently, the absorption of bone ceases and appositional growth of new bone occurs upon the walls of the cavities, and progresses until a longitudinal vascular canal of definitive size is reformed. The reversal from an erosive process to a formative process, which is thus inherent in the development of these units, is indicated by their demarcation from the surrounding tissues by the cement or reversal line which is seen as an unstained peripheral zone in Pl. 3, figs. 6 and 10. The preliminary erosive process involves not only the perivascular bone but also any Sharpey fibres which are included within it, and consequently, these fibres are never present in secondary osteones. The erosive process usually proceeds eccentrically from the original vascular canal, whereas the subsequent appositional growth of new bone tends to proceed concentrically. As a result the formation of a secondary osteone usually involves a shift in the position of the vascular canal concerned (Smith & Walmsley, 1959), and such a unit never contains more than one vascular canal except in the region of an anastomosis.

The first secondary osteones to appear in an area of bone develop in one of two different environments. They may form in association with the vascular canals included within surface bone during its formation (see above) and are then entirely surrounded by that tissue (Pl. 3, fig. 6). It may be noted, however, that although surface bone contains both longitudinal and radial vessels, secondary osteones form only in relationship with the former type. Furthermore, it has been observed that secondary osteones form with much greater facility in periosteal surface bone than in similar tissue of endosteal origin. Secondly, similar units may develop in relation to the vascular canals within primary osteones, and then they abut against both the remains of these primary osteones and the neighbouring woven-fibred bone (Pl. 3, figs. 7, 8).

Subsequent generations of secondary osteones develop in relation to the vascular canals of similar pre-existing units. Such an osteone may be bordered by the remnants of primary and secondary osteones and by woven-fibred bone (Pl. 3, fig. 8, *c*), or by the remnants of secondary osteones and by surface bone (Pl. 3, fig. 9). However, as more and more generations of new units are formed the amount of woven-fibred bone, primary osteones and surface bone is progressively reduced and there is a corresponding tendency for secondary osteones to become completely surrounded by the remnants of similar units (Pl. 3, fig. 10).

The fibre patterns in human secondary osteones have been described previously (Smith, 1959). In the present study no new fibre pattern has been noted, but it has been observed that in bone from a species other than man there is little variation in the structure of the secondary osteones, one fibre pattern being characteristic for the species. Thus in the radius of the horse the characteristic fibre pattern is that designated Type I, whereas in the metacarpus of the ox it is Type II.

Interstitial bone

Interstitial bone is a by-product of the development of secondary osteones. As secondary osteones are formed within a region of bone, parts of the pre-existing tissue become separated both from the bone surface and from the adjacent vascular canals by the reversal lines surrounding these units. These isolated regions are termed interstitial bone. It is evident from a consideration of the process by which secondary osteones are formed that interstitial bone may be of three different kinds. It may consist of the remains of woven-fibred bone and primary osteones (Pl. 3, fig. 8); secondly, it may consist of surface bone (Pl. 3, fig. 9), and thirdly, it may consist of the remnants of secondary osteones (Pl. 3, fig. 10).

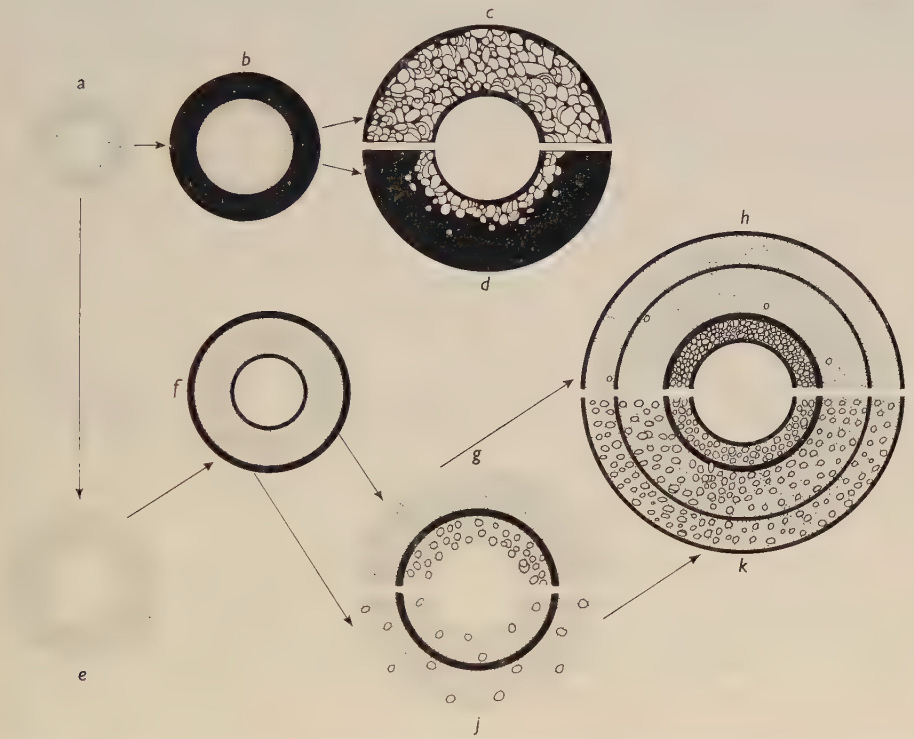
The relationship between the fibre pattern of a bone and its growth in girth

It is well known that, in general, the girth of the diaphysis of a long bone enlarges as a result of deposition of new bone on the periosteal surface and concurrent erosion of bone from the endosteal surface. The type of bone formed beneath the periosteum during growth varies in different species and at different stages of development, but always consists of either woven-fibred bone or surface bone.

After this subperiosteal bone has been formed, two changes occur in the internal architecture of the tissue—changes which, although they occur during growth, do not contribute in any way to that process. First, the vascular spaces in woven-fibred bone invariably become occupied by primary osteones soon after the tissue is formed. Secondly, secondary osteones are formed, by the process already described, in regions originally occupied by surface bone, or by woven-fibred bone and primary osteones. The time of onset of this change during development, and the rate at which it proceeds thereafter, varies in different species, in different bones of one species and even in different regions of one bone. The formation of secondary osteones usually begins long before growth is complete, but it does not cease when adult life is reached. On the contrary the process continues at a variable rate until death occurs.

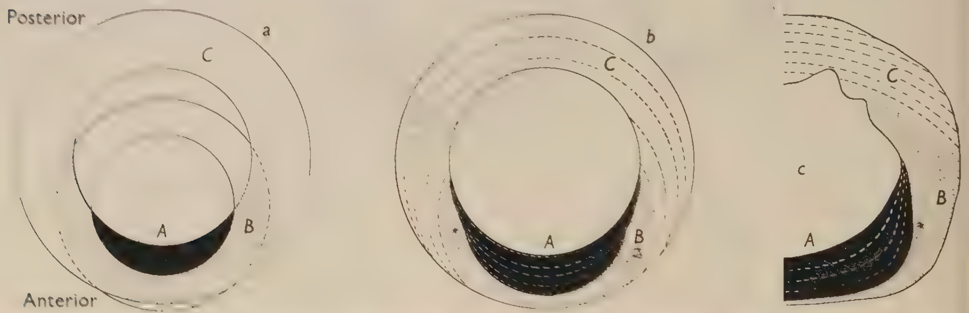
During life therefore a bone is affected by two distinct processes. The one is concerned with growth in the size of the bone and involves the production of woven-fibred bone and surface bone. The other does not contribute to growth in size: it is concerned with the internal architecture of the bone and involves the formation of primary and secondary osteones.

In the species examined during the present investigation, it has been observed that the maturation of long bones may proceed along two different lines of development and these are illustrated diagrammatically in Text-fig. 2. In the sequence *a*, *b*, *c*, *d*, the initial bone consists of woven-fibred bone in which primary osteones soon form (*a*). At an early stage of development, however, the nature of the subperiosteal deposition changes abruptly from woven-fibred bone to surface bone (*b*), and formation of this tissue continues thereafter until growth is complete. However, before the bone reaches its definitive size, endosteal erosion has removed all the original woven-fibred bone and primary osteones: moreover, secondary osteones have formed in relation to the longitudinal vascular canals within the surface bone (*c* and *d*). The adult bone thus consists of secondary osteones and surface bone together with interstitial bone which is derived from both of these tissues: the ultimate proportions of the various elements within the bone vary according to the rate of formation of secondary osteones. Where the rate is rapid (*c*) the bone may consist almost entirely of secondary osteones and interstitial bone except for thin marginal layers of surface bone on the periosteal and endosteal aspects. Such a pattern is exhibited by the transverse section of an adult cat femur in Pl. 4. fig. 1. On the other hand, when the rate is slow (*d*) secondary osteones may be confined to the innermost part of the bone, the greater part of the cortex consisting of unaltered surface bone. This structure is well illustrated in the transverse section through the tibia of a man of 68 years in Pl. 4, fig. 2. It was noted by Foote (1916) and has been confirmed by Amprino & Bairati (1936) that this mode of development is characteristic of human long bones.



Text-fig. 2. Stages in the development of mammalian long bones. Stipple represents woven-fibred bone and primary osteones; black represents surface bone; circles represent secondary osteones.

The other line of development is illustrated diagrammatically in Text-fig. 2 by the sequence *a*, *e*, *f*, *g*, *h*, *j*, *k*. The original model of woven-fibred bone continues to grow by deposition of woven-fibred bone and the formation within that tissue of primary osteones (*e*). Subsequently (*f*) the bone becomes coated on its periosteal and endosteal aspect by thin layers of surface bone. Thereafter, however (*g* and *j*) the endosteal layer becomes eroded as the marrow cavity enlarges, and simultaneously, the subperiosteal accretion reverts to woven-fibred bone. As a result a layer of surface bone becomes sandwiched between two regions of woven-fibred bone and primary osteones. The same process may be repeated so that additional layers of surface bone become similarly enclosed (*h* and *k*). The deposition of woven-fibred bone then continues until the adult size is reached, when thin final layers of surface bone are added to the endosteal and periosteal surfaces (*h* and *k*). Secondary osteones usually begin to develop while growth is in progress. In some situations these units form first in the older bone near the endosteal surface and spread outwards towards the periosteum (*g* and *h*) but in others they occur diffusely throughout the width of the bone (*j* and *k*). The number of secondary osteones varies considerably, even in different regions of the same bone. This mode of development is characteristic of horse, ox and dog. Thus Pl. 4, fig. 3, illustrates the posterior part of the radius of the adult horse. This region consists in large part of woven-fibred bone and primary osteones but also contains two included layers of surface bone and a few diffusely scattered secondary osteones. On the other hand, Pl. 4, fig. 4, shows the anterior part of the same bone in which secondary osteones are the predominant structural unit. They are evenly distributed throughout the bone and lie amongst the remains of the woven-fibred bone and primary osteones of which the region originally consisted.



Text-fig. 3. Eccentric diaphyseal growth. *a* and *b*, growth of rat femur (after Tomlin *et al.* 1953); *c*, tracing of transverse section of dog femur in Pl. 4, fig. 6. The interrupted lines in *b* and *c* indicate the orientation of collagenous formations.

The anterior part of the femur of a 13-year-old dog is illustrated in transverse section in Pl. 4, fig. 5. The outer part of the section consists of a periosteal layer of surface bone, two included layers of surface bone, circumferential primary osteones and woven-fibred bone. In the inner part, on the other hand, the original constitution of the tissue is now masked by numerous secondary osteones of the first and second generations.

Certain features additional to those already described may be found in those bones in which diaphyseal growth occurs eccentrically rather than concentrically. Thus Tomlin, Henry & Kon (1953) have demonstrated by auto-radiographic studies that increase in girth of the femoral diaphysis in the rat occurs in the manner illustrated in Text-fig. 3*a*. As the bone grows from the size indicated by the smaller annulus to that indicated by the larger, the posterior part of the bone extends by periosteal accretion and simultaneous endosteal erosion. The latter process eventually destroys the posterior part of the original bone. Anteriorly, on the other hand, the bone enlarges by endosteal accretion and periosteal erosion. The larger bone thus consists of three elements, namely an area of endosteal bone (black *A*), part of the original bone model (stippled *B*) and a large area of new periosteal bone (white *C*).

This mode of development accounts for certain features which are occasionally observed in mammalian long bones (Text-fig. 3*b*). Thus the area of endosteal bone (*A*) is always in the form of a crescent occupying the inner part of one side of the diaphysis, and in the material examined in the present investigation it has always consisted of surface bone. Moreover, as would be expected from its mode of formation the lamellae in the central part of the area lie parallel to the bone surfaces, whereas those on either side of the area tend to be directed obliquely from the endosteal towards the periosteal surface. The area of new periosteal bone (*C*) forms a larger crescent on the opposite side of the diaphysis and has a greater tendency than the endosteal crescent to occupy the whole width of the bone. Initially, it may consist either of surface bone or of woven-fibred bone in which primary osteones quickly form. But secondary osteones appear to develop in this area more rapidly than in the endosteal crescent so that the original character of the tissue may not be readily apparent. However, when the periosteal crescent consists of surface bone or of woven-fibred bone and circumferential primary osteones (p. 332), the lamellae, or the primary osteones as the case may be, lie parallel to the bone surfaces in the centre of the area, but again follow an oblique course from the endosteal to the periosteal surface on either side.

The area of original bone (*B*) consists of two wedge-shaped areas which intervene on either side between the periosteal and endosteal crescents and which are usually joined together behind the latter area. This is, of course, the oldest part of the definitive bone and is therefore the region most likely to be occupied by secondary osteones.

These three areas can be readily appreciated in the transverse section of the medial half of an adult dog's femur in Pl 4, fig. 6, and in the tracing of the section in Text-fig. 3*c*. The endosteal crescent (*A*) consists of surface bone and the inclination of the lamellae to the bone surface varies in the manner described above. The periosteal crescent (*C*) here consists of woven-fibred bone containing circumferential primary osteones and a few secondary osteones near the endosteal surface (see Pl. 4, fig. 5). And again the orientation of the primary osteones to the bone surfaces varies as they are traced from the centre to the side of the area. The area of original bone (*B*) extends in this case around the endosteal crescent and consists predominantly of secondary osteones.

In the transverse section of the femur of an adult cat (Pl. 4, fig. 8) the endosteal crescent of surface bone is readily apparent (see also Pl. 4, fig. 7) but because numerous secondary osteones have formed in the periosteal crescent the distinction between this area and that of original bone has been lost.

DISCUSSION

The terms which have been used in this paper to describe the various types of mammalian bone differ in some respects from those in common use. The simplest classification is the division of the tissue into primary and secondary bone, but unfortunately these terms are given different connotations by different authors. Thus for Lacroix (1951) the distinction between the two types lies in the time of their formation and their structure. Primary bone is found in the early stages of development and consists of what is described in this paper as woven-fibred bone, whereas secondary bone is formed during the later stages of development and consists essentially of the lamellated forms of the tissue. On the other hand, Fawcett (1954) regards primary bone as that formed in sites previously unossified and secondary bone as that formed in sites from which pre-existing bone has been absorbed. In this report Fawcett's definitions have been adopted. Another classification, which is derived from Weidenreich (1930), depends largely on the relationship which the collagen fibres of bone bear to one another. Thus bone is divided into woven-fibred bone in which the collagen fibres are arranged in a random fashion, parallel-fibred bone in which the fibres all run parallel to one another in the same direction, and lamellar bone in which the fibres are arranged in layers, the fibres of each layer having a specific orientation. The recognition of bone in which there is a random arrangement of the fibres and that in which there is an orderly arrangement is of value, but the distinction between parallel-fibred and lamellar bone, while academically correct, appears to serve no useful purpose, as it gives little indication of the developmental history of an area of bone.

This difficulty may be overcome, in part, by further subdividing parallel-fibred and lamellar bone into units or regions which are characterized by their developmental histories and the relationship of their collagen bundles to intra-osseous blood vessels. Thus osteones (Haversian systems) are those regions of bone in which the fibres are arranged in some concentric fashion around one or more vascular canals; whereas circumferential lamellar systems are those regions in which the fibres run parallel to the endosteal or periosteal surfaces of the bone and bear a tangential relationship to neighbouring vascular canals. It is evident, however, from the present investigation that there are two types of osteones—those forming *de novo* in the previously unossified vascular spaces of woven-fibred bone, and those whose formation is preceded by erosion of regions of pre-existing bone. Various terms have been employed to indicate this distinction. Thus osteones forming *de novo* have been designated primitive, simple, embryonal and atypical Haversian systems. Similarly, the first osteones to be formed in erosion cavities are regarded by some as the first generation of typical osteones and by others as the second generation of Haversian systems (Lacroix, 1951; Pritchard, 1956; Rouiller, 1956; Fawcett, 1954; Bloom, 1957).

Because the two types of osteones are distinct in both development and structure it seems reasonable to give definite recognition to this distinction in the terminology. The simplest way in which this can be done is to describe those osteones forming *de novo*, as primary osteones and to regard the units forming in erosion cavities as the first, second and third generation etc. of secondary osteones.

Again the term circumferential lamellar systems is inaccurate because the tissue concerned is by no means invariably lamellated and it is suggested that a more comprehensive term would be surface bone.

In this report, therefore, mammalian bone has been classified into four types, namely woven-fibred bone, primary osteones, secondary osteones and surface bone.

Interstitial bone consists of the remnants of the other four tissues. The main features of these tissue types are summarized in Table 1.

At the present time a large amount of experimental work is necessarily performed on the skeletal systems of lower mammals. Little regard is sometimes taken, however, of the different types of bone which are thereby being utilized, and very little is known of their particular properties or of their reaction to experimental procedures. It seems therefore of the greatest importance that the structure of the bones of an experimental animal should be clearly appreciated and that this structure should be taken into account when conclusions are drawn from experimental work.

The main stages through which the structure of a bone passes during its development are as much part of the inherent growth pattern as the shape of the definitive bone itself. On the other hand, it seems probable that further investigation may elucidate such factors as the incidence and rate of some of these changes. Thus it has been stressed by D'Arcy Thompson (1942) that in many mammals the rate of growth shows seasonal variations which are the results of changes in temperature and dietary conditions. Furthermore, it is well known that periosteal and endosteal layers of surface bone form when growth is slowing down towards its final completion. It may be, therefore, that the thin layers of periosteal surface bone which are periodically formed upon, and then included within, predominantly woven-fibred bones (Text-fig. 2) are associated with periods of diminished growth rate.

Table 1

	Woven-fibred bone	Primary osteones	Surface bone	Secondary osteones
Primary or secondary	Primary	Primary	Primary or Secondary	Secondary
Limitation by cement line	−	−	− +	+
Presence of Sharpey fibres	+	+	+ −	−
Arrangement of collagen bundles	Random	Orderly	Orderly	Orderly
Presence of lamellation	−	− +	− +	− +
Relationship of collagen bundles	Non-specific	Concentric	Tangential	Concentric

The rate of formation of secondary osteones in different parts of single bones is another problem which requires further investigation. The incidence of these units in the anterior and posterior parts of the radius of an adult horse of approximately twelve years (Pl. 4, figs. 3, 4), and in the human tibia and femur in old age (Pl. 3, figs. 9, 10) suggest that neither age of bone nor any hormonal influence is the predominant factor in stimulating their formation. Neither does the distribution of secondary osteones appear to be directly related to the attachments of muscles or ligaments. On the other hand, it is possible that the rate of formation of these units may be determined by the stress to which a given area of bone is normally subjected. The problem of stress distribution in individual bones is a difficult one which has received comparatively little attention, but the introduction of photoelastic techniques (Milch, 1940; Jessop & Harris, 1949; Fessler, 1957) and the application of resistance strain gauges to the examination of fresh bone (Hirsch & Brodetti, 1957) may be expected to give more information on this important subject.

SUMMARY

1. The fibre patterns in bone from man, ox, horse, sheep, pig, dog and cat have been examined in sections stained by Weidenreich's method and in ground sections viewed by polarized light.
2. A classification of bone types is suggested which is based on the developmental history of the area, its fibre pattern and the relationship of the fibre pattern to vascular canals.
3. The fibre patterns in four types of bone are described, namely woven-fibred bone, primary osteones, surface bone and secondary osteones.
4. The relationship of these bone types to the mechanism of growth in girth is discussed.

I have to thank Prof. R. Walmsley for his advice throughout this investigation, and also Messrs J. Brown and R. Stuart of this Department for their technical assistance.

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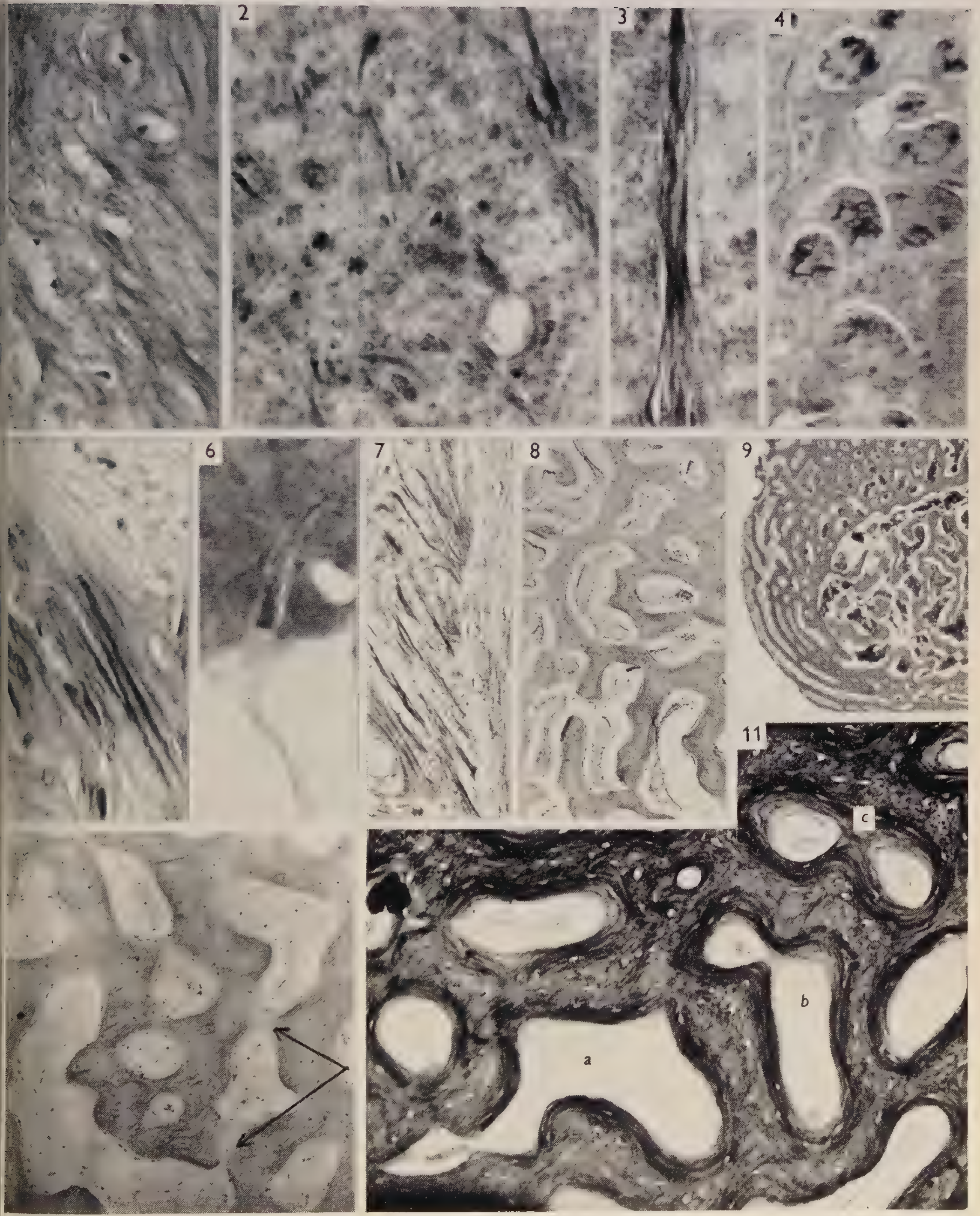

SMITH—COLLAGEN FIBRE PATTERNS IN MAMMALION BONE

(*Facing p.* 342)

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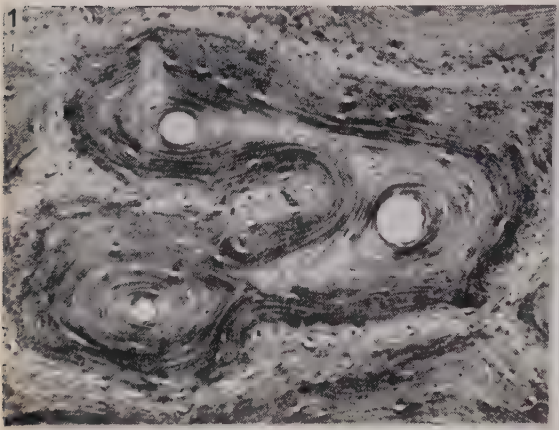

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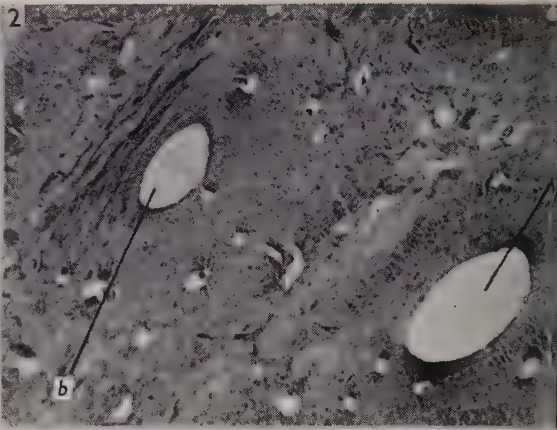

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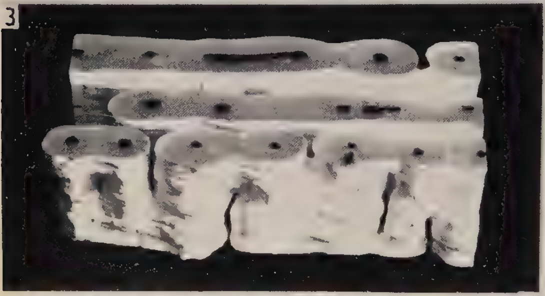

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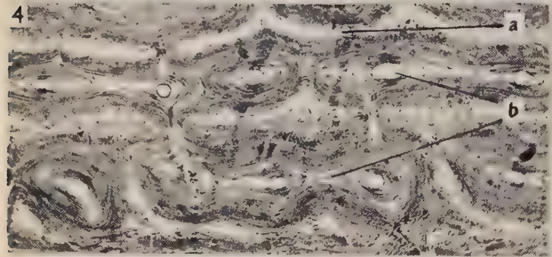

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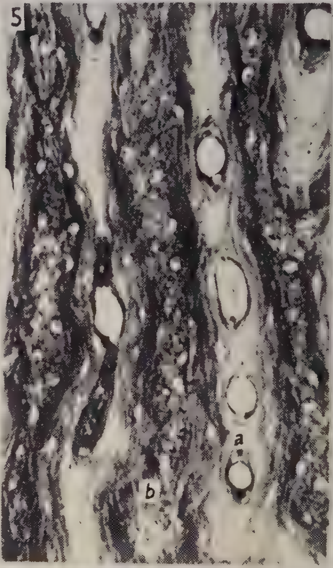

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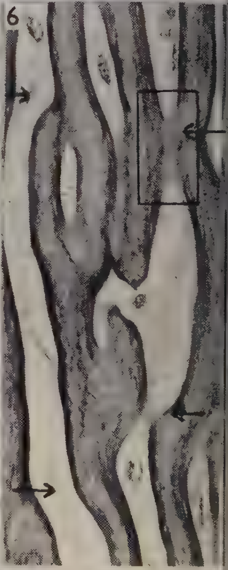

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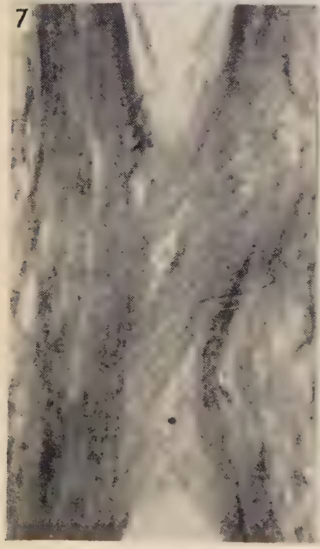

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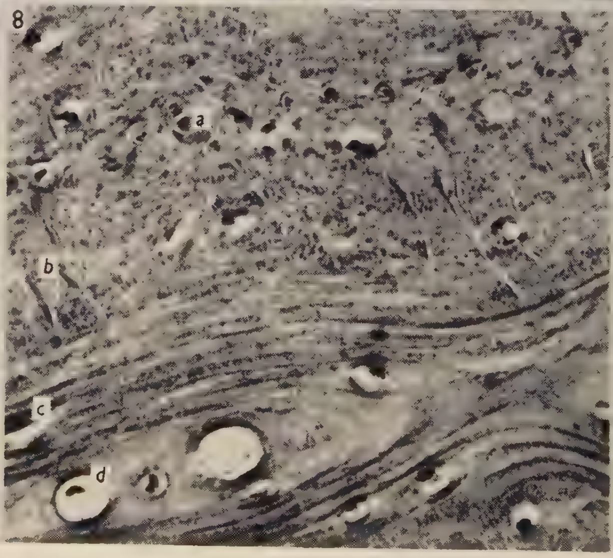

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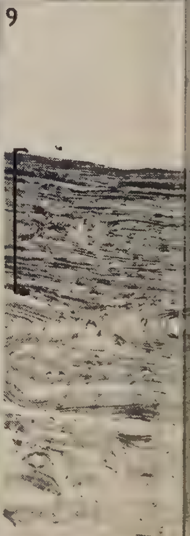

SMITH—Collagen fibre patterns in mammalian bone

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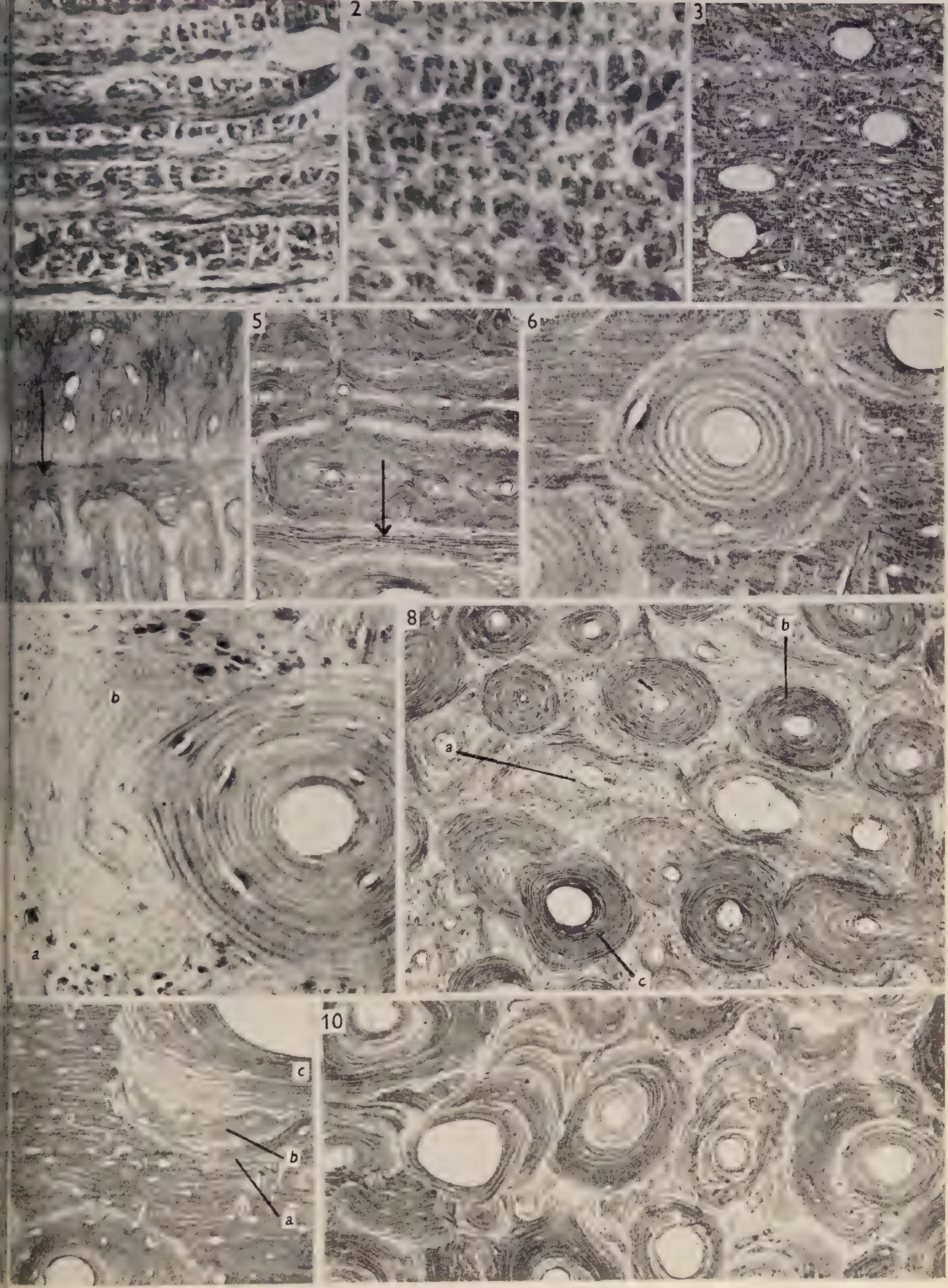
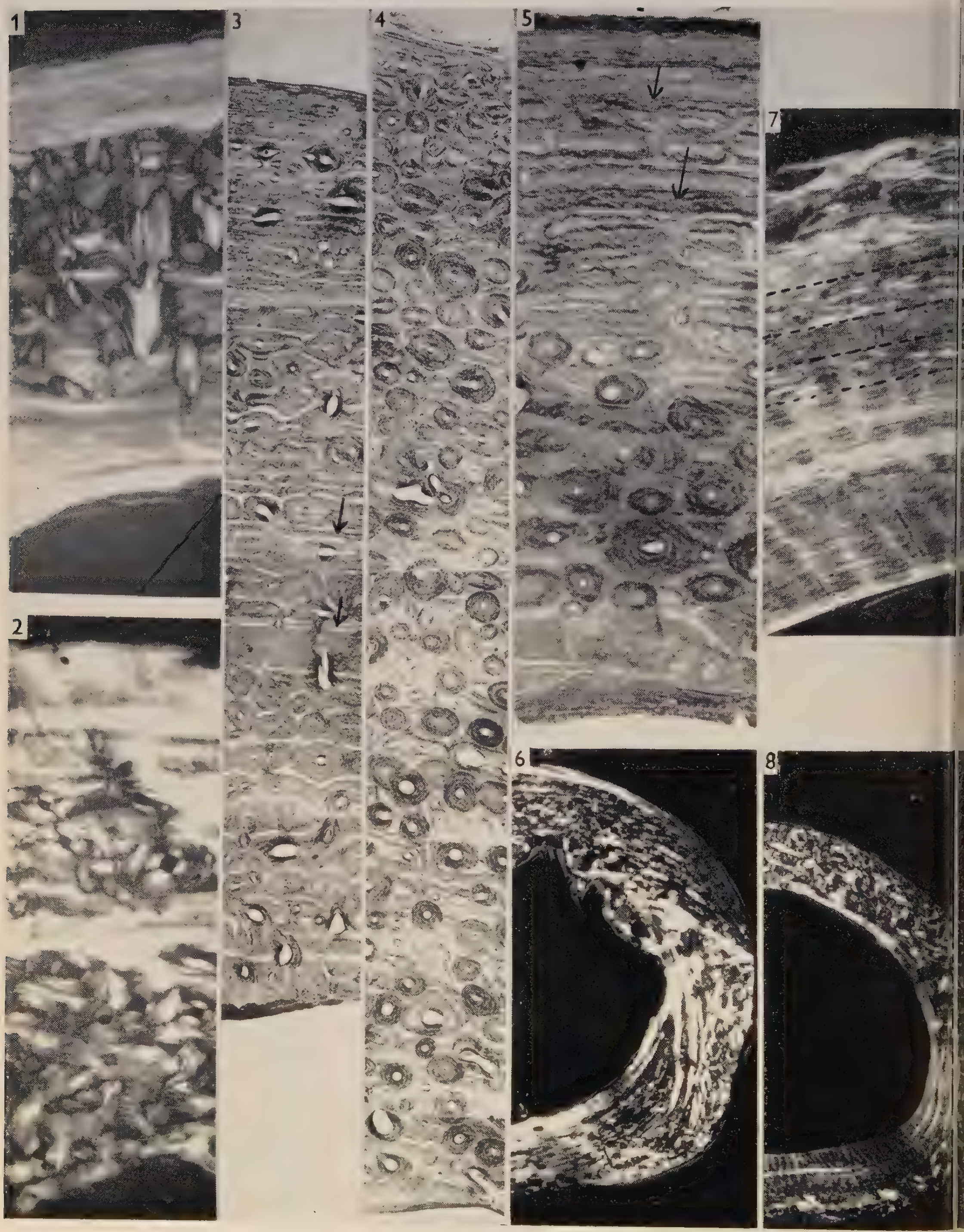
Plate 3



SMITH—Collagen fibre patterns in mammalian bone



SMITH—Collagen fibre patterns in mammalian bone

EXPLANATION OF PLATES

PLATE 1

Fig. 1. L.S. human tibia at birth. Woven-fibred bone. Weidenreich, × 500.
Fig. 2. T.S. human tibia at birth. Woven-fibred bone. Weidenreich, × 500.
Fig. 3. T.S. metacarpus of ox at 2 years. Sharpey fibre. Weidenreich, × 900.
Fig. 4. T.S. femur of 170 mm. human foetus. Sharpey fibres. Weidenreich, × 900.
Fig. 5. T.S. periosteal surface of human tibia at birth. Bone below, soft tissues above. Weidenreich, × 150.
Fig. 6. T.S. metacarpus of 7 months ox foetus. Bone above, intra-osseous vascular space below. Weidenreich, × 600.
Fig. 7. L.S. radius of adult horse. Secondary osteone on right. Weidenreich, × 150.
Fig. 8. T.S. metacarpus of 7 months ox foetus. Weidenreich, × 70.
Fig. 9. T.S. femur of 170 mm. human foetus. Posterior aspect above. H. & E., × 17.
Fig. 10. T.S. metacarpus of 7 months ox foetus. Arrows indicate pre-osseous bridges. Weidenreich, × 120.
Fig. 11. T.S. metacarpus of ox at 2 months. Weidenreich, × 150.

PLATE 2

All sections stained by Weidenreich's method

Fig. 1. T.S. radius of adult horse. Irregular primary osteone surrounded by woven-fibred bone. × 150.
Fig. 2. T.S. human tibia at 5 months. *a* and *b*, cylindrical primary osteones surrounded by woven-fibred bone. × 250.
Fig. 3. Wax plate reconstruction of the primary osteones from the radius of the adult horse, seen in fig. 4.
Fig. 4. T.S. radius of adult horse. The upper and lower margins of the figure are parallel to the periosteal surface. *a*, woven-fibred bone; *b*, circumferential primary osteones. × 65.
Fig. 5. T.S. metacarpus of ox at 2 years. The upper and lower margins of the figure are parallel to the periosteal surface. *a*, radial primary osteone; *b*, woven-fibred bone.
Fig. 6. L.S. human tibia at 5 months. Arrows indicate sites of deflection on the predominantly longitudinal course of the vascular canals of cylindrical primary osteones. × 60.
Fig. 7. The area outlines in fig. 6. × 300.
Fig. 8. T.S. radius of adult horse. *a*, woven-fibred bone containing Sharpey fibres. *b*, *c*, *d*, circumferential primary osteone. *b*, non-lamellated zone; *c*, lamellated zone; *d*, amorphous zone.
Fig. 9. T.S. periosteal surface, radius of adult horse. Bracket indicates surface bone. × 65.

PLATE 3

All sections stained by Weidenreich's method

Fig. 1. T.S. adult human tibia. Lamellated surface bone. × 800.
Fig. 2. T.S. adult human femur. Non-lamellated surface bone. × 800.
Fig. 3. T.S. adult human tibia. Outer part of cortex. Lamellated surface bone, traversed by Sharpey fibres. × 80.
Fig. 4. T.S. metacarpus of ox at 7 years. The arrow indicates an included layer of non-lamellated surface bone. × 70.
Fig. 5. T.S. radius of adult horse. The arrow indicates an included layer of lamellated surface bone. × 65.
Fig. 6. T.S. adult human tibia. First-generation secondary osteone in surface bone containing Sharpey fibres. × 200.
Fig. 7. T.S. radius of adult horse. *a*, woven-fibred bone containing Sharpey fibres; *b*, cylindrical primary osteone; *c*, first-generation secondary osteone. × 360.
Fig. 8. T.S. radius of adult horse. *a*, circumferential primary osteone surrounded by woven-fibred bone; *b*, first-generation secondary osteone; *c*, second-generation secondary osteone. × 65.

Fig. 9. T.S. adult human tibia. *a*, *b* and *c*, first-, second- and third-generation secondary osteones in surface bone. × 200.

Fig. 10. T.S. adult human femur. Secondary osteones in interstitial bone derived from similar unit. × 110.

PLATE 4

Fig. 1. T.S. femur of adult cat. Periosteal surface above. Ground section. Polarized light, × 40.

Fig. 2. T.S. medial part of adult human tibia. Periosteal surface above. Ground section. Polarized light, × 30.

Fig. 3. T.S. posterior part of radius of adult horse. Periosteal surface above. Arrows indicate included layers of surface bone. Weidenreich, × 20.

Fig. 4. T.S. anterior part of radius of adult horse. Periosteal surface above. Weidenreich, × 20.

Fig. 5. T.S. anterior part of femur of adult dog. Periosteal surface above. Arrows indicate included layers of surface bone. Weidenreich, × 50.

Fig. 6. T.S. right half of femoral shaft of adult dog. Anterior aspect above. See text-fig. 3*c*. Ground section. Polarized light, × 8.

Fig. 7. T.S. posterior part of femur of adult cat. Periosteal surface above. The interrupted lines enclose the two circumferential zones of longitudinal vascular canals. Ground section. Polarized light, × 40.

Fig. 8. T.S. right half of femur of adult cat. Anterior aspect above. Ground section. Polarized light, × 8.

VARIATIONS IN THE METATARSAL BONES

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A study of the accounts of the metatarsal bones given in standard text-books of Anatomy shows that few variations in these bones are described. More or less identical descriptions of these bones are given by Brash (1951), Breathnach (1958), Frazer (1940), Johnston, Davies & Davies (1958), and Wood Jones (1949), in the respective text-books edited by them. Examination of the bones, however, reveals frequent variations from the typical description.

This paper presents the results of a study of 100 specimens of each of the metatarsal bones. It is not considered necessary to give a detailed description of each bone as the descriptions referred to above are adequate in most respects. This account is, therefore, confined to a description of the variations encountered. There are no significant differences in the findings in bones of the right and left sides and hence these are not described separately.

OBSERVATIONS

First metatarsal

The proximal articular surface of this bone is generally described as a kidney-shaped area with the hilum on the lateral side. In the present investigation, a typical kidney-shaped area is seen only in 15 specimens. In the majority (60 specimens), the medial margin of the surface is slightly indented. This encroachment on the articular area is marked in 25 specimens, thus producing two partially united, upper and lower, facets (Fig. 1*a*). This appearance is accentuated, in some specimens by the presence of a transverse ridge that runs across the surface in this situation. This ridge may occur even in the absence of a notch in the medial margin. The proximal surface is usually gently concave. When the transverse ridge is present, however, the concavity is no longer continuous, there being separate upper and lower concave areas.

The lateral side of the base of the first metatarsal shows a smooth facet, with well-defined margins for the second metatarsal, in 21 specimens (Fig. 1*b*). In 40 specimens it is represented by a smooth area with indefinite margins (Fig. 1*c*). No indication of an area for the second metatarsal is seen in the remaining 39 bones.

The plantar aspect of the head of the bone shows a prominent ridge separating medial and lateral grooves for sesamoids. The medial groove is invariably wider and also deeper. The lateral part is prolonged proximally on the plantar aspect of the shaft, more than the medial.

According to Wood Jones, the nutrient foramen to this bone is always on the lateral side of the shaft. In the present series the position of the nutrient foramen, or foramina, is as follows.

(i) On the lateral aspect of the shaft in 62 specimens.

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(ii) On both lateral and medial aspects in 15 specimens.
(iii) On lateral and dorsal aspects in 7 specimens.
(iv) On lateral and plantar aspects in 2 specimens.
(v) On medial aspect alone in 2 specimens.
(vi) On dorsal aspect alone in 1 specimen.
(vii) On plantar aspect alone in 11 specimens.

The foramina are sometimes two or more even when confined to one surface of the shaft. In some cases the foramen is very large, out of proportion to the size of the bone. These features are also seen in other metatarsals.

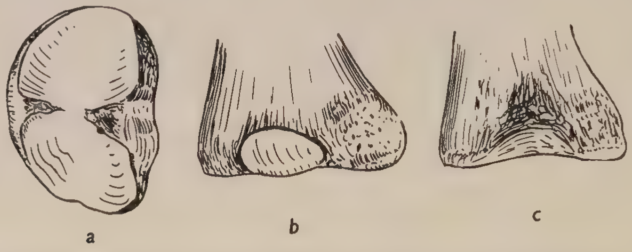

Fig. 1. (*a*) Proximal surface of the first metatarsal showing partial subdivision of the articular area into upper and lower parts (*b*), (*c*) Lateral side of the base of the first metatarsal showing a definite facet (*b*), and a smooth area (*c*), for the base of the second metatarsal.

Second metatarsal

The proximal triangular surface for the intermediate cuneiform invariably shows a distinct concavity, more marked towards the lateral side.

The medial side of the base of this bone usually shows a facet on its dorsal part, for the medial cuneiform bone. This facet is very variable in size. The proximal part of the facet is either flat or slightly convex and this may be the only part present (Fig. 2*a*). The distal part, when present, is gently concave (Fig. 2*b*). The facet generally extends up to the proximal margin of the base to become continuous with the facet for the intermediate cuneiform (Fig. 2*a*, *b*). It is, however, partially cut off from this area by a non-articular notch in 8 specimens, and is completely cut off in 4 specimens (Fig. 2*c*). The facet is absent in 8 specimens, but one of them shows a pressure facet in this situation.

More inferiorly and distally, the medial side of the base of the second metatarsal shows a definite smooth facet, with well-defined margins, for the first metatarsal, in 12 specimens (Fig. 2*c*). An elevated area, often rough is present at this site in 57 specimens (Fig. 2*a*). The remaining 31 bones show no indication of contact with the first metatarsal (Fig. 2*b*).

The facets on the lateral side of the base of this bone may assume numerous patterns.

(*a*) In 49 specimens there are two, dorsal and plantar, facets separated by a non-articular area. The proximal parts of both facets are bevelled off. The distal areas, for the third metatarsal are always larger and better defined than the proximal areas, for the lateral cuneiform, the latter being generally small (Fig. 2*d*).

(*b*) In 22 specimens, the plantar facet is not bevelled off and articulates only with the third metatarsal (Fig. 2*e*).

(*c*) Six specimens show only the proximal bevelled part of the plantar facet, there being no plantar facet for the third metatarsal (Fig. 2*f*).

(*d*) The entire plantar facet is absent in 9 specimens (Fig. 2*g*).

(*e*) In three specimens, the entire plantar facet and the proximal part of the dorsal facet are absent, a non-articular strip taking the place of the latter (Fig. 2*h*).

(*f*) In 5 specimens, the proximal bevelled part of the dorsal facet is alone absent. Again it is replaced by a non-articular strip (Fig. 2*i*).

(*g*) In one specimen the proximal bevelled parts of both plantar and dorsal facets are absent. The plantar facet reaches the proximal margin of the base to become continuous with the area for the intermediate cuneiform, but the dorsal facet is separated from the latter by a non-articular area (Fig. 2*j*).

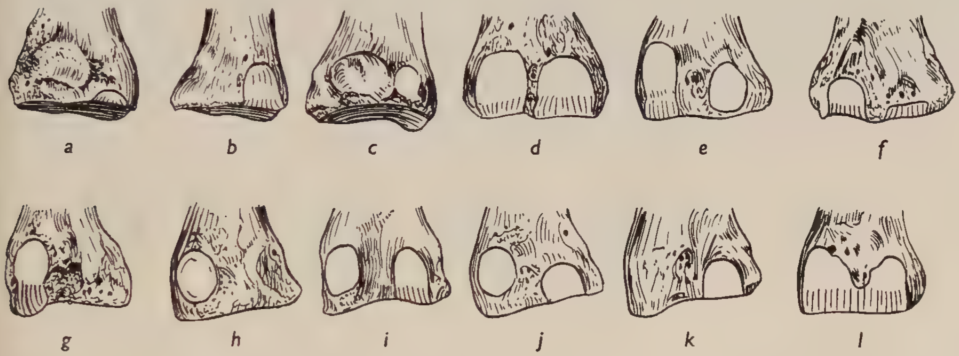

Fig. 2. (*a–c*). Various forms of the facets on the medial side of the base of the second metatarsal, for the base of the first metatarsal and the medial cuneiform. (*d–l*) Various forms of the facets on the lateral side of the base of the second metatarsal, for the third metatarsal and the lateral cuneiform.

(*h*) In another specimen, the plantar facet is similar to that described in (*g*) above, but the entire dorsal facet is missing (Fig. 2*k*).

(i) Finally in 4 specimens, the plantar and dorsal areas for the lateral cuneiform are continuous with one another, those for the metatarsal remaining separate (Fig. 2*l*).

Grant (1958), in a figure illustrating the various forms of the facets on the lateral side of the base of the second metatarsal, depicts a condition wherein both plantar and dorsal facets for the cuneiform are present, but those for the metatarsal are absent. No such specimen is seen in the present series.

The proximal parts of the facets, for the lateral cuneiform, are always flat, but the distal parts, for the third metatarsal, are occasionally somewhat concave.

According to Wood Jones the nutrient foramen to this bone is always situated on the lateral aspect of the shaft. In the present investigation the position of the foramen, or foramina is seen to be as follows:

(i) On the lateral aspect of the shaft in 60 specimens.
(ii) On the medial aspect in 16 specimens.
(iii) On the dorsal aspect in 3 specimens.
(iv) On both the lateral and medial aspects in 6 specimens.

(v) On both lateral and dorsal aspects in 7 specimens.
(vi) No distinct foramen is present in 8 specimens.

Third metatarsal

The proximal triangular articular surface for the lateral cuneiform bone is very constant in shape. It is always flat, in contrast to the concavity of the proximal surface of the base of the second metatarsal. In fact, this feature alone frequently suffices to distinguish the two bones from one another.

The medial side of the base of this bone shows two flat facets, plantar and dorsal, for the base of the second metatarsal, in 57 specimens (Fig. 3*a*). The facets reach the proximal margin of the base to become continuous with the articular surface for the lateral cuneiform. The dorsal facet is always the larger of the two. The plantar facet is at times barely perceptible. Both plantar and dorsal facets are absent in 2 specimens. The plantar facet is absent alone in 41 specimens (Fig. 3*b*); in two of them the dorsal facet does not reach the proximal margin of the base from which it is separated by a non-articular strip (Fig. 3*c*).

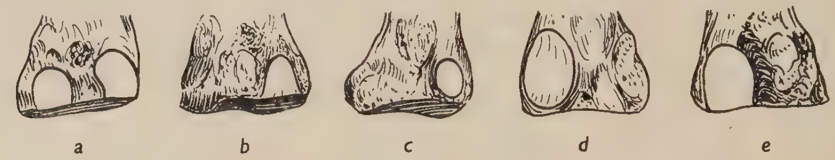

Fig. 3. (*a*, *b*, *c*) Various forms of the facets on the medial side of the base of the third metatarsal, for the second metatarsal. (*d*, *e*) Various forms of the facet on the lateral side of the base of the third metatarsal, for the fourth metatarsal.

The lateral side of the base of this bone presents a very constant facet on its dorsal part for the fourth metatarsal. This facet is always conspicuous. In 36 specimens, it is oval and does not reach the proximal margin of the base (Fig. 3*d*). In the remaining 64 specimens it reaches that margin to become confluent with the area for the lateral cuneiform, and the facet has the appearance of an oval with its proximal part cut off (Fig. 3*e*). The facet is usually concave but may be flat.

The shaft of this bone usually shows a slight convexity towards the medial side. Usually, it also shows a twist so that the long axis of the proximal articular surface of the base, and that of the articular surface of the head, are not in the same plane. The dorsal aspect of the head is carried medially and the plantar aspect laterally.

In 100 third metatarsal bones examined by him, Wood Jones found that the nutrient foramen is situated on the lateral side in 73 bones, and on the medial side in 27 bones. In the present series, the position of the nutrient foramen, or foramina, is as follows.

(i) On the lateral side of the shaft in 53 bones.
(ii) On the medial side in 25 bones.
(iii) On both lateral and medial sides in 13 bones.
(iv) On the plantar aspect in 2 bones.
(v) On the dorsal aspect in 1 bone.
(vi) No distinct foramen is seen in 6 bones.

Fourth metatarsal

The medial side of the base of this bone usually shows an oval, flat or gently concave facet. Rarely, the facet is convex or concavo-convex. It is usually described as being subdivided by a vertical ridge into a proximal part for the lateral cuneiform and a distal part for the third metatarsal. The illustration in *Gray's Anatomy* (Johnston, Davies & Davies), shows the facet to be separated from the proximal margin of the base by a distinct non-articular area. On the other hand, Wood Jones states that the area for the cuneiform is inconstant, but when present it reaches the proximal margin of the base to become continuous with the area for the cuboid. In the present study three varieties of the facet are seen. In 61 specimens, there is an oval facet not reaching the proximal margin of the base, and not subdivided into two parts (Fig. 4*a*). The entire facet is for the metatarsal, there being no facet for the cuneiform. In 22 specimens the facet is again oval, not reaching the proximal margin of the base, but is subdivided into two parts (Fig. 4*b*). In 15 specimens the facet reaches the proximal margin to become continuous with the articular area for the cuboid. It is subdivided into proximal and distal parts (Fig. 4*c*).

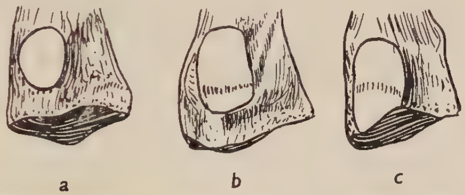

Fig. 4. Various forms of the facet on the medial side of the base of the fourth metatarsal, for the third metatarsal and the lateral cuneiform.

The proximal part of the facet, when present, is generally small. In 2 specimens no facet is seen on the medial side of the base of the bone.

The lateral side of the base of the fourth metatarsal shows a concave, somewhat triangular facet. It is always present and varies only slightly in size and shape. A constant deep notch is present in front of it.

The shaft of this bone shows a convexity towards the medial side, more marked than that of the third metatarsal but less marked than that of the fifth. It also shows a more marked twist than the third metatarsal.

In 100 fourth metatarsal bones examined by him, Wood Jones found that the nutrient foramen is on the lateral side in 60 bones and on the medial side in 40. In the present series the position of the foramen, or foramina, is as follows.

(i) On the medial side of the shaft in 44 bones.
(ii) On the lateral side of the shaft in 36 bones.
(iii) On the dorsal aspect of the shaft in 4 bones.
(iv) On both medial and lateral sides in 8 bones.
(v) On both lateral and dorsal sides in 1 bone.
(vi) On both medial and dorsal sides in 1 bone.
(vii) On medial, lateral and dorsal sides in 1 bone.
(viii) No distinct foramen is seen in 5 bones.

Fifth metatarsal

The fifth metatarsal bone shows few variations from the typical description. The shaft of the bone generally shows a well marked convexity to the medial side.

The facets for the fourth metatarsal and for the cuboid are constant in size and shape.

According to Wood Jones the nutrient foramen to this bone is always on the medial side. In the present series the position of the foramen, or foramina, is as follows:

(i) On the medial side in 75 specimens.
(ii) On the medial and dorsal sides in 11 specimens.
(iii) On the medial and lateral sides in 5 specimens.
(iv) On the medial and plantar sides in 3 specimens.
(v) On the medial, dorsal and plantar sides in 1 specimen.
(vi) On the plantar aspect alone in 2 specimens.
(vii) On the dorsal aspect alone in 1 specimen.
(viii) No distinct foramen is present in 2 specimens.

SUMMARY

1. The variations encountered in 100 specimens of each of the metatarsal bones have been studied.

2. The facets on the sides of the bases of the metatarsal bones show frequent variations from the typical text-book description. Variations are most commonly seen on the lateral side of the base of the second metatarsal. No significant variations are seen on the fifth metatarsal and on the lateral side of the fourth.

3. The site of entry of the nutrient artery (or arteries) into the bones is also subject to considerable variation.

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THE EARLY DEVELOPMENT OF THE NASAL CAVITY AND UPPER LIP IN THE HUMAN EMBRYO

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INTRODUCTION

The general pattern of facial and nasal development is well known. The two regions are so intimately associated that, especially in the early stages, the development of one cannot be considered apart from that of the other. Our knowledge of their embryology is based upon the classical work of Wilhelm His (1892, 1901), which was carried out in the closing decades of the last century. This has been supplemented and extended by the studies of Hochstetter (1891, 1892, 1944, 1950), Peter (1913, 1950), Frazer (1911, 1931), Boyd (1933), Streeter (1948), Politzer (1951–3) and others. Where relevant their views will be summarized and discussed later in this paper, but it should be pointed out here that Peter's Atlas of 1913 provides a most extensive and detailed review of the whole field.

While there is substantial agreement between the various investigators, divergences of view upon some points still exist. Certain interesting descriptions put forward by Frazer have been widely accepted by British embryologists and because of their important theoretical implications it seemed desirable that they should be re-examined. The main contentions of Frazer that will be studied in the present investigation are:

(I) That the maxillary process grows across the open hinder end of the nasal pit and fuses with the fronto-nasal process thus converting the nasal pit into a primitive nasal cavity by making a new 'floor' for its hinder part, at the same time marking off the anterior naris and the site of the primitive posterior naris. Subsequently the maxillary process overgrows and buries the lower end of the fronto-nasal process. Eventually the two maxillary processes meet and fuse in the mid-line, thus providing a basis from which the whole of the upper lip later develops, the lower end of the fronto-nasal process having been completely excluded from the surface.

(II) That a layer of maxillary mesoderm, which he terms the 'septal process', spreads medially from the maxillary process across the roof of the primitive stomatodaeum and replaces the paraxial mesoderm of this region. The 'septal processes' of the two sides meet in the mid-line and they provide the mesoderm for a part of the roof of the nasal cavity and for a large portion of the nasal septum.

MATERIAL AND METHODS

Serial sections of a series of human embryos, ranging in size from 7 to 22 mm. crown-rump length, were used. The sections were examined microscopically and a wax plate reconstruction of the ventral part of the head of each embryo was made.

In all fifteen embryos were employed. In the description that follows, the embryos have been arranged according to their degree of development and not according to their size, as it was found in several instances that these did not correspond.

OBSERVATIONS

7·5 mm. embryo

In order to avoid confusion, in the account which follows, a plane passing through the nasal pits parallel to the long axes of the mandibular processes will be taken as running directly dorso-ventrally and one at right angles to this as going rostro-caudally.

In this embryo the broad opening of the stomatodaeum is bounded caudally by the mandibular arch, laterally by the maxillary processes and rostrally by the prominent, overhanging front part of the head. A wide shallow concavity in the roof of the stomatodaeum continues forwards and upwards over the front of the head. On either side of this the head bulges out into smooth convex elevations. The maxillary processes are small, being only slightly raised above the general surface level, and the surface grooves which mark the boundaries between the processes and the adjoining parts of the head are shallow and can only be identified with difficulty. In all there is little to indicate the future appearance of the face (Pl. 1, fig. 1).

Although the surface contours of the model offer no indication of their presence, microscopical examination of the sections reveals the existence of the nasal placodes. These oval-shaped areas of thickened epithelium are situated one on each side, on the lateral bulges of the head, and are ventral to the maxillary processes. They face laterally, caudally and ventrally and their long axes runs caudally and somewhat medially. They are about 400 μ long, 300 μ in width and 45 μ in thickness. The placodal epithelium is between four and five cells thick and at the margins of the placode there is a sharp transition between its epithelium and the much thinner epithelium that surrounds it. Many mitotic figures are present in the most superficial layer of the placodal epithelium (Pl. 1, fig. 2).

The surface epithelium is separated from the fore-brain by a layer of mesoderm, which for the most part is of a loose texture. Mainly this layer is narrow but deep to the placode it broadens out and the mesoderm immediately adjacent to the placode is condensed—its cells are closely packed and many of them are undergoing division (Pl. 1, fig. 2). The mesoderm of the maxillary process, also, is condensed and proliferating. The maxillary mesoderm is in direct contact medially with the loose paraxial mesoderm of the roof of the stomatodaeum and above with the loose mesoderm that extends over the side of the head (Pl. 1, fig. 3).

7–10 mm. embryos

In all, six embryos measuring between 7 and 10 mm. were studied. Despite the variations in size they have all reached about the same stage of development and therefore will be described together as a group.

The nasal placodes have 'sunk' below the general surface level and have been incorporated into the nasal pits. These, which are separated from each other by the wide central concavity seen in the previous embryo, are situated far out laterally on the ventral aspect of the head (Pl. 3, fig. 11). The pits, which are oval in outline, are narrow from side to side and have long axes which run caudally and slightly medially. They open ventrally, caudally and laterally. Each pit consists of a ventral open part which is directly continuous with a dorsally placed pouch or pocket which ends as a cul-de-sac. This dorsal part is the 'Hinteren Blindsack' of Peter (1913) and will be referred to here as the 'blind sac'. The open part is bounded by a raised up edge which forms the lateral and medial nasal processes, which are continuous with each other round the rostral end of the pit (Pl. 1, fig. 4; Pl. 3, fig. 11). The medial nasal process is more prominent than the lateral, extending farther ventrally and caudally. Its enlarged lower or caudal end is the globular process of His. The two medial nasal processes (including the globular processes) and the concave area between them form the fronto-nasal process.

The 'blind sac' is very shallow, being about 100μ in depth. Its medial wall is formed by the medial nasal process, while the rostral part of the lateral wall is formed by the lateral nasal process. This wall is completed caudally by the maxillary process which extends forwards below the lateral nasal process and to the lateral side of the enlarged lower end of the medial nasal process (Pl. 1, fig. 5). It should be clearly understood that the 'blind sac' is blind in the sense that it does not open caudally into the stomatodaeum. It communicates freely with the ventral part of the pit and the division between the two must be arbitrarily set. The 'blind sac' should be regarded as the dorsal part of the pit which is closed off caudally from the stomatodaeum by the apposition of the maxillary and medial nasal processes.

The boundaries between the various facial processes are formed by shallow surface grooves. These processes do not have long freely projecting ends. Their epithelium passes without break or interruption from the surface of one process to that of the next, while the mesoderm of one process is, for the most part, quite freely in contact with that of adjacent processes. The border between the maxillary process and the lateral nasal process is provided by the naso-lachrymal groove. The boundary groove between the maxillary process and the medial nasal process begins at the caudal end of the nasal pit. At first it runs for a short distance downwards across the ventral aspect of the head and then turning on to the roof of the stomatodaeum it passes backwards and medially towards Rathke's pouch (Pl. 3, fig. 12). Beyond the dorsal limit of the medial nasal process the groove marks the border between the laterally placed maxillary process and medially that part of the roof of the stomatodaeum which is formed from paraxial mesoderm.

The paraxial mesoderm of the roof of the stomatodaeum is of a loose texture. Ventrally it is continuous with the mesoderm of the fronto-nasal process and laterally it is in contact with the more condensed mesoderm of the maxillary process. As judged by the differences in density between the maxillary and paraxial mesoderms, it is clear that the maxillary mesoderm is limited to the maxillary process and there is no indication of it spreading medially into the territory of the paraxial mesoderm (Pl. 1, fig. 6). That is, there is no evidence at this stage of the 'septal process'. Ventral to this the maxillary mesoderm is separated from the fronto-nasal mesoderm of the medial nasal process partly by the nasal pit and partly by an epithelial sheet which extends from the caudal margin of the 'blind sac' to the boundary groove between the maxillary and fronto-nasal processes (Pl. 1, fig. 5). This sheet which was originally described by Hochstetter (1891, 1892) and has been given the English name of 'nasal fin' (Streeter, 1948), is continuous both with the epithelium of the 'blind sac' and that of the surface. In specimens of the present group in which the 'blind sac' is shallow the nasal fin is correspondingly short. The epithelium of the fin contains mitotic figures. The ventral part of the boundary groove to which it is attached has been named the primitive palatine groove (Peter, 1913).

14 *mm. embryo A* (Pl. 3, fig. 13)

This embryo has been compressed a little from side to side, but if the slight resultant distortion is disregarded, the general appearance of the model shows little change from that of the previous group. The relationships of the facial processes to each other remain unaltered and the boundary grooves between them can be easily identified. However, the 'blind sac' has increased considerably in size and its full extent cannot be seen in the model as its dorsal end is hidden from view, but examination of the sections shows it to be about 500μ long. Marked changes have occurred to the nasal fin. The ventral end of the fin has disappeared and this has allowed the mesoderms of the maxillary and medial nasal processes to come into contact, caudal to the blind sac, over a distance of about 110μ. Although these mesoderms are in contact there is no evidence of spread of maxillary mesoderm into the fronto-nasal process. Behind this zone the fin persists and here it shows some indication of the cavitation which results in the further deepening of the pit and the formation of the bucco-nasal membrane.

12·5 *and* 13 *mm. embryos*

In the previous embryos there was little indication of the face as such, but in these its outlines are more easily recognizable, although it is still overhung by the bulging front part of the head. The mesoderm of the face has increased greatly in amount and there is a thick mesodermal layer between the forebrain and the roof of the mouth. The nasal pits are closer together. No longer far out laterally on the ventral aspect of the head, they are flanked externally by large lateral nasal processes, while medially the central concavity between the relatively smaller medial nasal processes is beginning to be flattened out. Despite these changes, the relationships of the facial processes round the nasal pits remain unaltered and the boundary grooves are still distinct (Pl. 4, fig. 15).

The nasal pit is deeper and the 'blind sac' is now about 700μ long. The ventral part of the nasal fin has disappeared over a distance of approximately 530μ, thus allowing a greater area of contact between maxillary and fronto-nasal mesoderms, but no indication of medialward spread of maxillary mesoderm could be detected (Pl. 2, fig. 7). Dorsal to this the fin remains. This part of the fin exhibits marked cavitation, several large spaces being present in it (Pl. 2, fig. 8). In its most dorsal part the fin has been transformed into a thin flattened sheet of cells—the bucco-nasal membrane (Hochstetter, 1891, 1892)—which is now all that separates the 'blind sac' from the cavity of the stomatodaeum (Pl. 2, fig. 9).

15 *mm embryo*

The building up of the face which was apparent in the last embryo has continued so that the facial region is no longer overhung by the front part of the head. The facial processes are still recognizable, although the boundary grooves between them are becoming less distinct. Their relationships to each other continue to remain the same and there is no indication of the maxillary process overgrowing the fronto-nasal process. The nasal pits are closer together and their ventral openings are smaller (Pl. 3, fig. 14).

The 'blind sacs' are deep. On the right side the bucco-nasal membrane persists but on the left side it has ruptured thus establishing continuity between the nasal pit and the stomatodaeum (Pl. 4, fig. 16). The pit on this side has now given rise to the primitive nasal cavity; its original opening forms the anterior naris and the new opening produced by the breakdown of the bucco-nasal membrane is the primitive posterior naris or primitive choana. The primitive posterior naris is small and it is at the dorsal end of the primitive palatine groove.

14 *mm. embryo B*

The appearance of this embryo is very similar to that of the previous specimen. The processes still maintain their original relationships to each other and the grooves between them are very shallow. The primitive palatine groove is better marked in the roof of the stomatodaeum than on the ventral aspect of the face but it can still be traced to the caudal end of the anterior naris. At the dorsal end of each primitive palatine groove there is a well-formed primitive posterior naris which is overhung on its lateral side by a small palatal process (Pl. 4, figs. 17, 18).

14 *mm. embryo C*

The face is much larger in this specimen and the nasal region projects forwards like a snout from below the front of the head. The anterior nares are closer together. The primitive posterior nares have enlarged particularly in a dorso-ventral direction and the palatine processes have grown correspondingly in size.

The grooves between the facial processes are less distinct than in the previous embryo but the primitive palatine groove can still be traced forwards from the primitive posterior naris. On the roof of the stomatodaeum it is fairly clear but on the ventral aspect of the face, where it crosses the region of the presumptive upper lip, it is barely detectable. Nevertheless, the groove continues in this region to mark the boundary between the maxillary and fronto-nasal processes and the original relationship between these processes stays unchanged (Pl. 5, figs. 19–20).

16 *mm. embryo*

The model of this embryo shows the snout-like region of the face. The groove between the maxillary and fronto-nasal processes has disappeared both on the ventral aspect of the face and the roof of the mouth. With the disappearance of this groove the boundary between these processes has vanished and one is now only justified in speaking of the region that was maxillary or that was fronto-nasal process. The separation of the upper lip from the rest of the upper jaw region has not yet commenced, and there is no evidence of the labio-dental groove (Pl. 2, fig. 10).

22 *mm. embryo*

In this specimen the upper lip can now be recognized. The labio-dental groove has appeared and it extends evenly across the buccal surface of the upper jaw region. There is no indication that the lip develops in two separate halves which later fuse in the mid-line.

DISCUSSION

Development of the primitive nasal cavities

On the basis of the observations reported here and those recorded in the literature it is apparent that in the early development of the nasal cavities four stages can be recognized. The first stage is characterized by the formation of the nasal placodes, in the second stage the placodes give rise to the nasal grooves, while in the third stage the nasal grooves are converted into the nasal pits and in the fourth the pits deepen and the primitive posterior nares are formed. Embryos in the present series provide examples of the first, third, and fourth stages. The transformation of a nasal placode into a nasal groove was thought by Peter (1913) to be brought about by active growth of the placodal epithelium. Kallius (1905), on the other hand, suggested that the epithelium remained passive and that the changes in contour were produced by proliferation of the mesoderm deep to the edge of the placode. Possibly both processes play a part, but whatever the mechanism, the placode 'sinks' below the general surface level and becomes bounded by a raised up edge or rim which is deficient caudally (Keibel, 1912). The structure thus formed is the nasal groove, and according to His its lower end is narrow and extends caudally to open directly into the stomatodaeum. This narrow caudal portion was called the naso-buccal channel by Keith (1948) and he compared it with the communication that exists between the mouth and nasal cavity in the fully developed dogfish. The nasal pit is formed according to His (1892, 1901) by the maxillary process growing medially across the open naso-buccal channel and fusing with the medial nasal process, thus providing a floor to the pit and at the same time demarcating the open anterior and primitive posterior nares. Following Hochstetter's (1891, 1892) description of the bucco-nasal membrane, this account was modified a little and the primitive posterior naris was described as being temporarily occluded by an epithelial plug.

Peter (1913) and Keibel (1893, 1912) also describe the conversion of the nasal placodes into the nasal grooves. Both agree with Hochstetter (1891, 1892) and Della Vedova (1907) that the grooves never open directly into the stomatodaeum and there is thus no naso-buccal channel across which the maxillary process can grow in the way indicated by His. If such a channel existed the maxillary process could only extend across it by virtue of possessing a freely projecting ventral end. His regarded this as being the case, as indeed the term process, which he used, implies, but Peter (1913) and Streeter (1948) have pointed out the fallacy of this view and their criticisms are supported by the present material. As indicated in the description of the embryos of the 7–10 mm. group there is no evidence of the processes having freely projecting ends. The epithelium extends unbroken from the surface of one process to that of another and in general the mesoderms of adjacent processes are in contact. Streeter believed that the processes are in reality growth centres and that their surface elevations and bulges are produced by proliferation of the underlying mesoderm. The boundaries between the processes are indicated by surface grooves which later become obliterated by the proliferation of the mesoderm deep to them and as this occurs the processes appear to unite. As Peter observed this takes place without the fusion of the epithelial edges of the grooves and so-called 'epithelial fusion zones' do not exist. Indeed one looks in vain for any evidence of an 'epithelium of fusion' between, say, the two medial nasal processes when these coalesce to form a single mass.

If the views expressed in the previous paragraph on the structure of the nasal groove and the nature of the facial processes be accepted, it becomes more difficult to explain how exactly a nasal groove changes into a nasal pit. Keibel (1912) and Peter (1913) (the latter in spite of his views on the nature of the facial processes) state that the maxillary process makes contact and then fuses with the medial nasal process. The fusion, taking place from within outwards, occurs as soon as the processes come into contact with each other. They thus regard the nasal fin as an 'epithelium of fusion' between the maxillary and medial nasal processes. In the present paper it is suggested that the nasal fin is better considered, not as an 'epithelium of fusion', but as a structure that plays an important role in the deepening of the nasal pit and the formation of the primitive posterior naris. As already pointed out, the nasal groove is probably formed and deepened by the proliferation of mesoderm round its edges. At the same time the maxillary mesoderm is increasing in amount and because of this the maxillary process extends farther and farther ventrally. Eventually the maxillary process reaches the nasal groove and comes to form its wall caudo-laterally. When this has occurred the groove has been converted into the nasal pit and the maxillary process then forms the caudal part of the lateral wall of the 'blind sac', as shown by the 7–10 mm. embryos. The nasal groove is bounded by an elevated rim which forms the lateral and medial nasal processes, and the nasal pit differs from the groove in having the maxillary process form part of its wall and in the presence of the 'blind sac'. As already indicated in the description of the embryos, in the earlier stages the 'blind sac' does not open into the stomatodaeum, and writers such as Keibel and Peter employed the term in order to emphasize this point. It is retained here, for the same reason, to stress that the nasal pits are at first shut off from the stomatodaeum. As the nasal pit develops the maxillary process extends ventrally caudal to the lateral nasal process and to the lateral side of the medial nasal process. It is suggested that as this takes place epithelium may become trapped between the ventral end of the maxillary process and the medial nasal process thus giving rise to the nasal fin. On the other hand, Streeter (1948) is of the view that the fin is formed entirely by epithelial proliferation and he writes that 'the epithelium exhibits a characteristic growth activity'. Certainly many mitotic figures are present within the cells of the fin, and proliferation must play a considerable part in its growth but it seems doubtful that it is completely responsible for the fin's formation.

Once the nasal pit and nasal fin have been established the course of events which constitute the fourth of the developmental stages, becomes clearer. At first, as the 'blind sac' deepens there is a corresponding increase in the length of the nasal fin. Next the ventral part of the fin ruptures and this allows maxillary and fronto-nasal process mesoderms to come into contact caudal to the pit, as has occurred in the 14 mm. embryo A. Whether the breakdown of this part of the fin is due to cell death or to a moving apart of the epithelial cells of the fin could not be determined in the material available. After this the more dorsal part of the fin appears to split along its length and this cleavage has the effect of deepening the 'blind sac'. This splitting is preceded by the development of cavities within the epithelium of the fin as shown in the 12·5 and 13 mm. embryos and the complete cleavage is presumably brought about by the coalescing of these cavities. The nasal fin is thus reduced in size as the 'blind sac' enlarges dorsally. Eventually the fin is represented by no more than a thin sheet of cells—the bucco-nasal membrane—which separates the 'blind sac' from the cavity of the stomatodaeum. Finally the rupture of the bucco-nasal membrane is brought about by the death and disintegration of the cells that form it. This results in the formation of the primitive posterior naris, through which the primitive nasal and mouth cavities now communicate. These views are in general agreement with those expressed by Peter who regarded the 'blind sac' as being deepened partly by mesodermal proliferation and partly by the splitting of the nasal fin. On the other hand, Streeter thought that the whole of the 'blind sac' was derived entirely by cleavage of the fin.

The development of the upper lip

The present observations support the views of the older anatomists such as His (1901), Keibel (1912) and Peter (1913) on the development of the upper lip. They regarded the lateral parts of the upper lip as being derived from the maxillary process, while the median portion arose from the fronto-nasal process. In this series of embryos the maxillary and fronto-nasal processes can be recognized as separate structures until the 14 mm. embryo C, but in the 16 mm. embryo the demarcating groove between these processes has been obliterated, and after this has occurred it is no longer possible to distinguish one process from the other. The upper lip cannot be identified in these embryos, and it is not until the development of the labio-dental groove in the 22 mm. embryo that its outlines become apparent. Although the upper lip does not arise until after the demarcating groove between the maxillary and fronto-nasal processes has disappeared, there can be no doubt that the median part of the lip arises from what was fronto-nasal process and the lateral parts from what were the maxillary processes. Once these processes come into relationship with each other, this relationship remains unchanged for as long as their identity can be recognized. The models show no evidence of the overgrowth of the fronto-nasal process by the maxillary processes in the manner described and illustrated by Frazer, nor is there any microscopical indication of medial spread of maxillary mesoderm into the fronto-nasal process.

Frazer (1931) implied that his description of the development of the upper lip explained its nerve supply. He appeared to believe that each major facial process or pharyngeal arch has its own specific nerve and that the nerve of a process supplies all the mesodermal derivatives of that process; that is, the nerve and mesoderm of a process are intimately 'tied together'. He thought that the whole of the upper lip was derived from maxillary mesoderm and that this accounted for its innervation by the infra-orbital nerve—a branch of the nerve of the maxillary process. In fact the median part of the upper lip arises from the fronto-nasal process, and its innervation by the infra-orbital nerve indicates that the nerve of a process can become disassociated from the mesoderm of the process and that the distribution of the nerve of a process in the adult does not furnish a reliable guide to mesodermal spread during embryonic development. A further example of the disassociation of the maxillary nerve and maxillary mesoderm is provided by the long spheno-palatine nerve. This branch of the maxillary nerve crosses the roof of the nasal cavity to reach the nasal septum, where it supplies a considerable portion of the mucous membrane. Frazer described a spread of maxillary mesoderm into the regions supplied by this nerve, giving the name of 'septal process' to this mesodermal sheet. The 'septal process' could not be detected during the present investigation; as has been pointed out no medial spread of maxillary mesoderm into the roof of the stomatodaeum could be found. Indeed the long spheno-palatine nerve is distributed to structures derived from paraxial and fronto-nasal process mesoderm.

Apart from the direct embryological evidence, cases of bilateral hare-lip provide data that indicate the separation of the maxillary nerve from maxillary mesoderm. It is generally conceded that in this condition the median tubercle is derived from the fronto-nasal process. Yet King (1954), in a study of the anatomy of hare-lip, based upon both dissected and sectioned material, has shown that the median tubercle, of which the median portion of the lip is a part, is supplied by the infra-orbital nerve which pursues an abnormal course to reach this destination.

In two recent papers Ashton & Oxnard (1958*a*, *b*) have described the distribution of the maxillary nerve in a large number of mammals, including many primates. They found that branches of the infra-orbital nerve supply the side and vestibule of the nose and, when present, the rhinarium. According to Boyd (1933) the rhinarium develops from the fronto-nasal process so that its innervation by the infra-orbital nerve provides a further example of a nerve leaving its 'own' mesoderm to enter and supply that of an adjacent process.

If nerve and mesoderm are intimately tied together, presumably during early development each mesodermal zone must exert some sort of special influence which causes only its 'own' nerve to enter and make connexions with it. In the case of maxillary mesoderm only outgrowing maxillary nerve fibres would be attracted and mesoderms of other origins would have no influence on these fibres. The experimental studies of nerve growth which are reviewed in several excellent papers by Weiss (1939, 1950, 1955) offer no support for this idea of the 'specific attraction' of outgrowing nerve fibres by particular regions of the embryo. Indeed it would seem that outgrowing nerve fibres are in no way 'attracted' by peripheral parts of the embryo, but that the direction in which they grow is determined by the orientation of the embryo's ultrastructure.

The development of hare-lip

It is usually stated (see Bremer (1957) and Willis (1958) for reviews) that hare-lip results from the failure of fusion of the maxillary and medial nasal processes, and that this is because the ventral ends of the processes never come into actual contact with each other. If, however, the description of early nasal and facial development put forward in this paper is accepted, another explanation emerges. It is suggested that development proceeds in the normal way with the formation of the nasal pit and the nasal fin, but that once formed the ventral part of the fin persists instead of disappearing to allow the maxillary and fronto-nasal process mesoderm to make contact. Subsequently the dorsal part of the fin undergoes the normal cavitation and cleavage, which results in the formation of the primitive posterior naris. An extension of this cavitation and cleavage into the abnormally persisting ventral part of the fin would result in the formation of a hare-lip. This explanation places a greater emphasis on the behaviour of the epithelium than is usual, but given that the ventral part of the fin persists, it seems reasonable to suppose that the cleavage which occurs as a normal event in the dorsal part of the fin should spread into it. It must, of course, be admitted that this explanation is speculative, as indeed the explanation of the origin of any congenital defect that is based upon normal material must be.

SUMMARY

1. The early development of the nasal cavities and the upper lip was studied in fifteen human embryos.

2. The development of the nasal pits is described and the part played by the 'nasal fin' in the deepening of a nasal pit and the formation of a primitive posterior naris is indicated.

3. The median part of the upper lip develops from the fronto-nasal process and the lateral parts from the maxillary processes.

4. No evidence in support of Frazer's view that the maxillary process overgrows the lower end of the fronto-nasal process or of his 'septal process' was found. The implications of this are discussed.

5. A mode of development of hare-lip involving the persistence of the 'nasal fin' is put forward.

I wish to thank Professors J. D. Boyd, G. Romanes and R. Walmsley for their kindness in allowing me to visit their departments and examine their collections of human embryos. I also wish to thank Prof. G. M. Wyburn for his encouragement and advice. I am indebted to Miss D. V. Henning for the drawings. The photographs are the work of Mr G. Marshall to whom I express my thanks.

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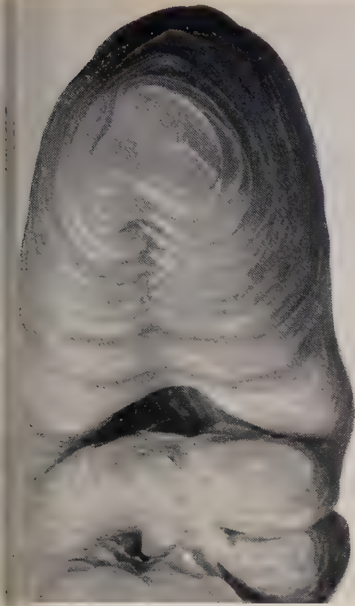
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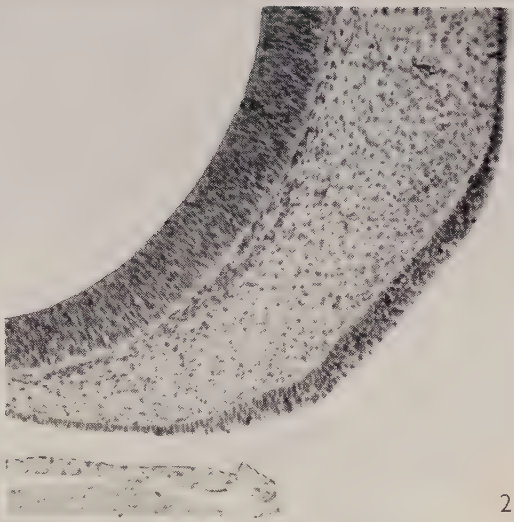
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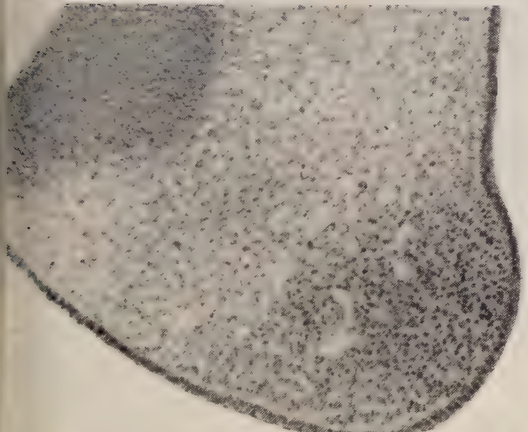
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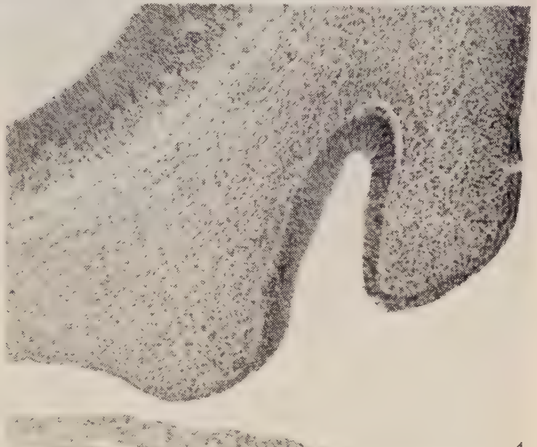
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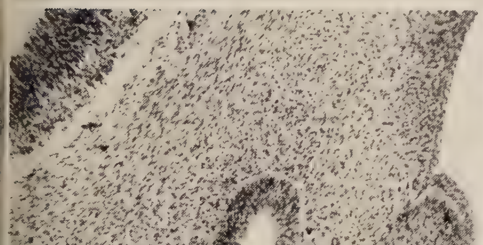
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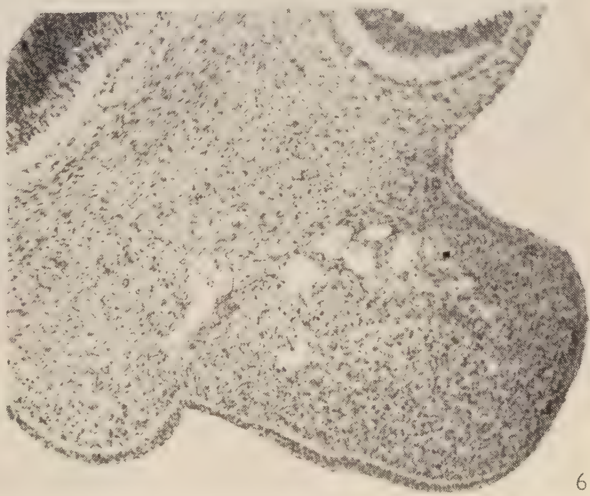
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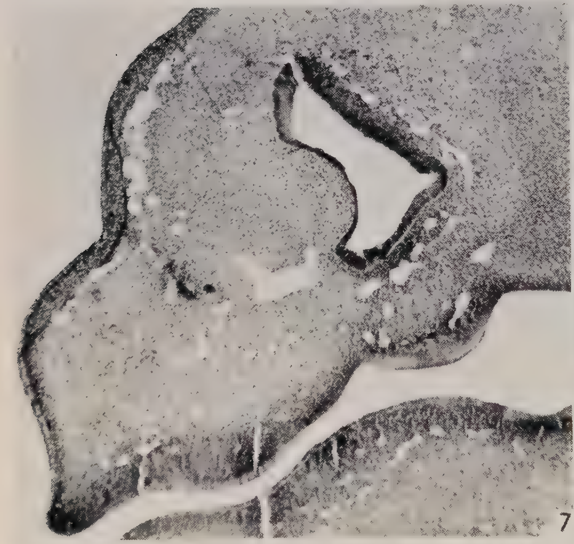
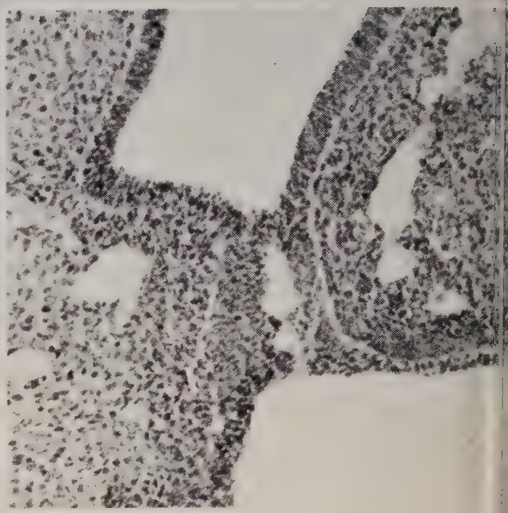
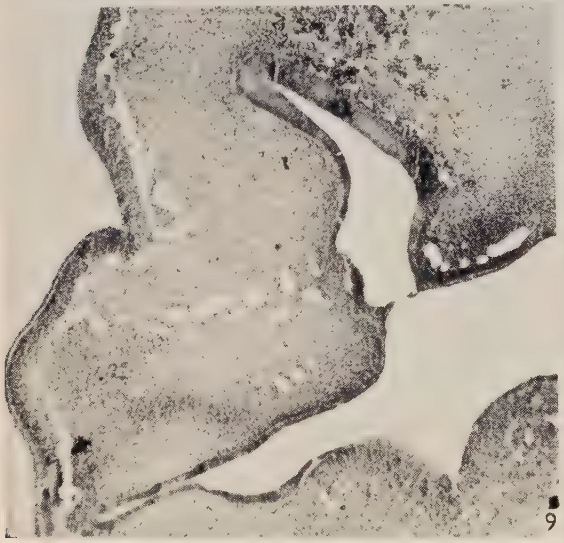
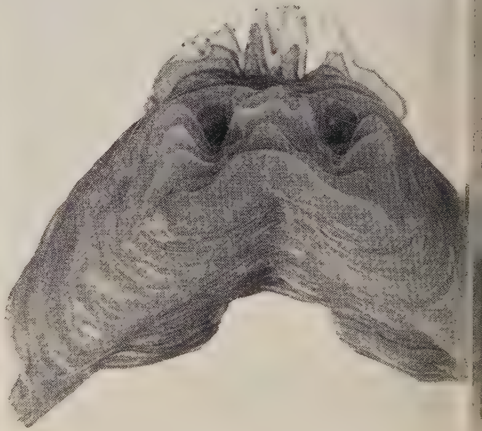
WARBRICK—Early development of the nasal cavity and upper lip

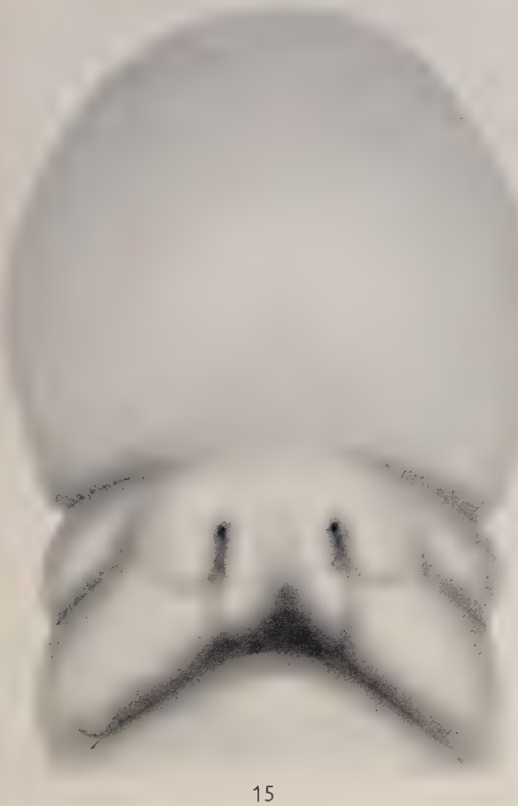








WARBRICK—EARLY DEVELOPMENT OF THE NASAL CAVITY AND UPPER LIP



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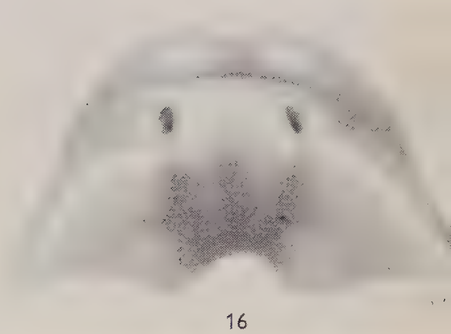

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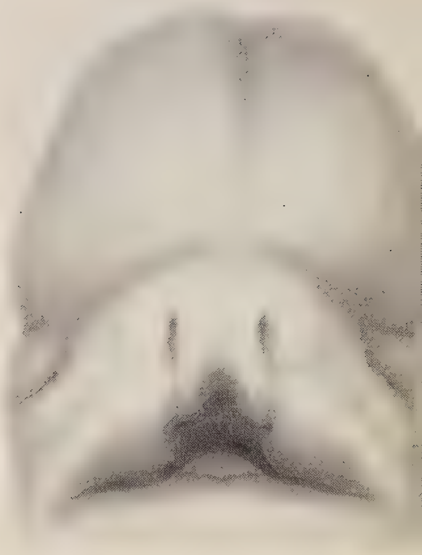

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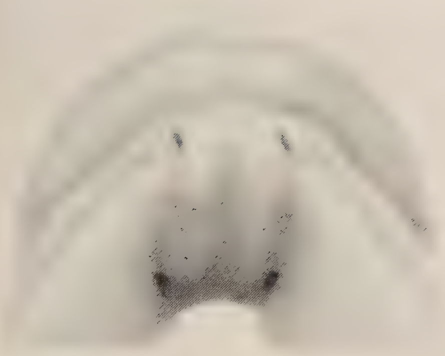

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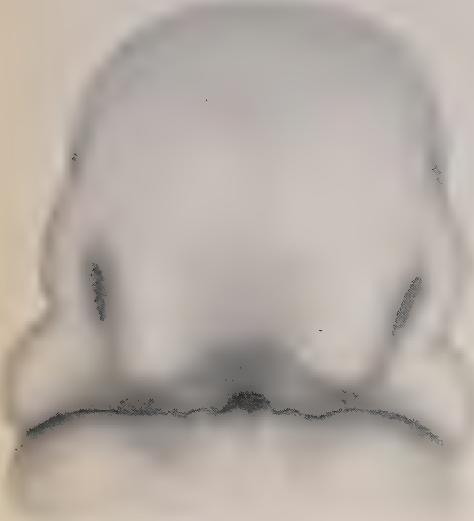
WARBRICK—Early development of the nasal cavity and upper lip



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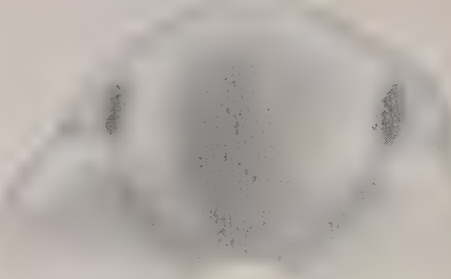

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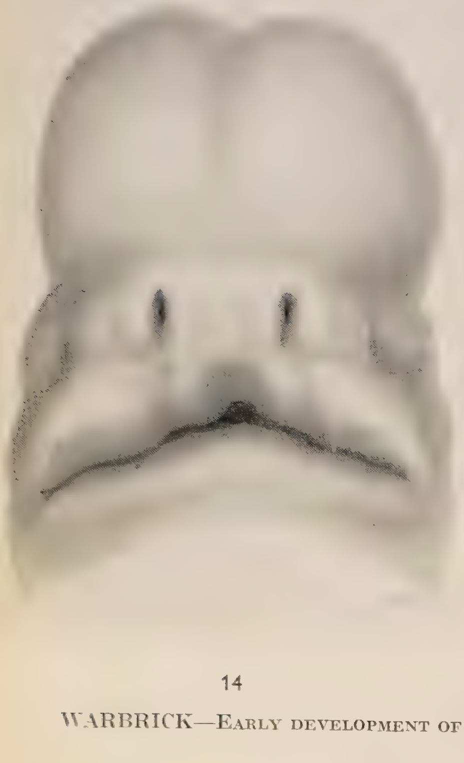

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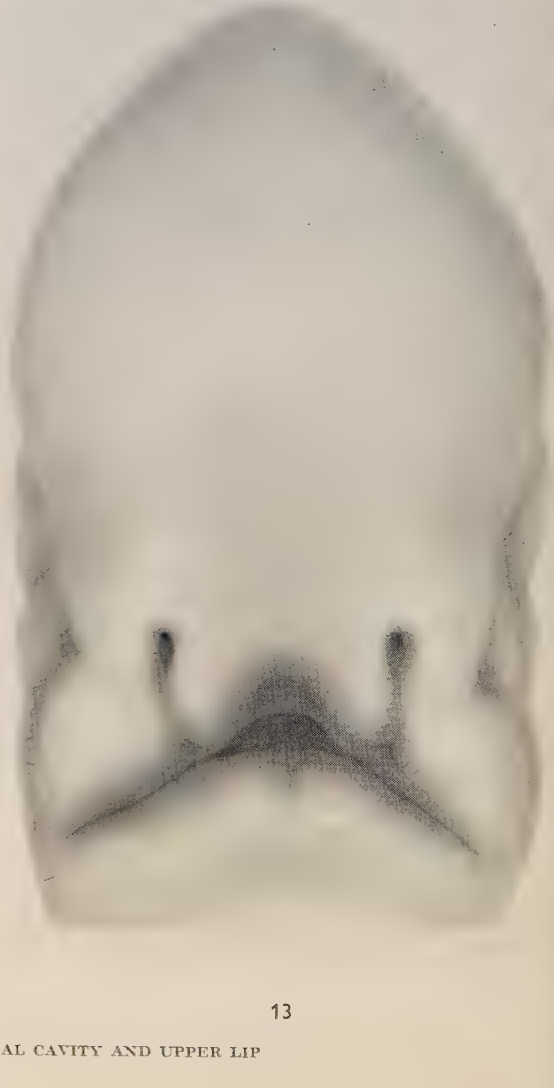

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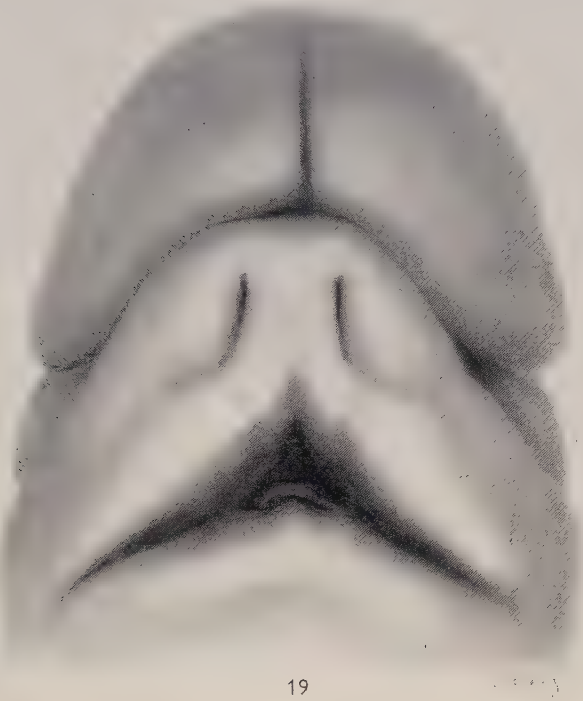

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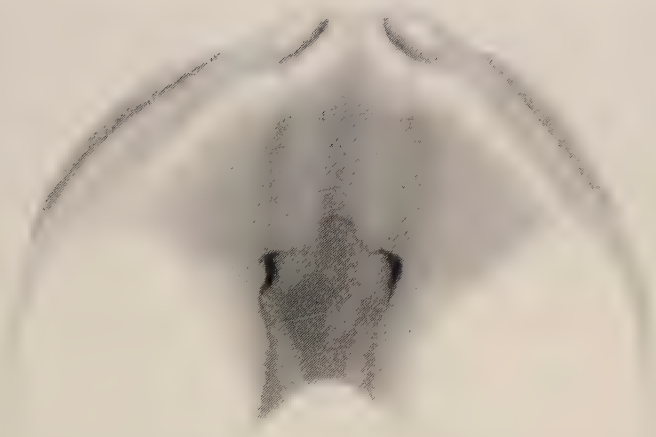

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photograph of the model of the head of the 7·5 mm. embryo showing the boundaries of the stomatodaeum. Note the small maxillary processes and the lateral convexities of the head.

Fig. 2. Transverse section through the head of the previous embryo showing a nasal placode. The mesoderm underlying the placode is condensed. × 57.

Fig. 3.] Section through the same embryo as in Fig. 2. Note the condensation of the mesoderm of the small maxillary process. × 57.

Fig. 4. Transverse section through the open part of a nasal pit of a 9·5 mm. embryo. × 95.

Fig. 5. Section through the previous embryo showing a 'blind sac' and 'nasal fin'.

Fig. 6. Section through the 9·5 mm. embryo. The relationship of maxillary to other mesoderms is shown. Note that the maxillary mesoderm is limited to the maxillary process and that there is no indication of its medial spread into the central part of the roof of the stomatodaeum. × 95.

PLATE 2

Fig. 7. Transverse section of the 12·5 mm. embryo showing the 'blind sac'. Note that the nasal fin has disappeared and that maxillary and fronto-nasal process mesoderms are in contact caudal to the 'blind sac'. × 57.

Fig. 8. Section through the previous embryo showing cavitation of the nasal fin. × 200.

Fig. 9. Section through the same embryo as in Fig. 8 showing the bucco-nasal membrane. × 57.

Fig. 10. Photograph of the model of the 16 mm. embryo. Note that the boundary grooves between the facial processes have now disappeared.

PLATE 3

Fig. 11. Drawing of a model of the 7 mm. embryo showing the ventral aspect of the head. The nasal pits with their surrounding processes can be seen widely separated from each other.

Fig. 12. Drawing of the model of the 7 mm. embryo showing the roof of the stomatodaeum. The boundary groove between the maxillary process and the central part of the roof of the stomatodaeum and more ventrally the caudal end of the medial nasal process is shown on each side.

Fig. 13. Drawing of the model of 14 mm. embryo A. The relationships of the facial processes round a nasal pit are the same as in the 7 mm. embryo.

Fig. 14. Drawing of the model of the 15 mm. embryo showing that the relationships of the facial processes are unchanged.

PLATE 4

Fig. 15. Drawing of the model of the 13 mm. embryo. Note that although the nasal pits are closer together the facial processes have the same relationships to each other round the pits.

Fig. 16. Drawing of the model of the 15 mm. embryo to show the roof of the stomatodaeum. The primitive posterior naris of the left side is shown opening in the boundary groove.

Fig. 17. Drawing of the model of the 14 mm. embryo B. Note the close similarity in appearance between this and the 15 mm. embryo.

Fig. 18. Drawing of the roof of the stomatodaeum of the previous embryo. The primitive posterior nares are present on both sides and lie in the boundary grooves.

PLATE 5

Fig. 19. Drawing of the model of 14 mm. embryo C. It is only just possible to identify the groove between the maxillary and fronto-nasal processes, which retain their original relationship to each other.

Fig. 20. Drawing of the roof of the stomatodaeum of the previous embryo. Note the shallow boundary groove with the primitive posterior nares lying in it. There is no indication that the maxillary process is overgrowing the caudal end of the fronto-nasal process.

THE INFLUENCE OF HEAT-STRESS ON THE PARS NERVOSA OF THE MOUSE

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Medical Research Council Climate and Working Efficiency Unit, Department of Human Anatomy, University of Oxford

INTRODUCTION

There is now a large body of evidence to suggest that the posterior pituitary hormones, or their precursors, are secreted by the paraventricular and supraoptic nuclei of the hypothalamus and transported down the axons traversing the hypophyseal stalk to the hypophysis where the antidiuretic hormone is released (Scharrer & Scharrer, 1954) in response to two main types of stimulus. One of these involves an increase in the osmotic pressure of the blood which provides the specific stimulus for the release of the antidiuretic hormone (Verney, 1946, 1947); the other has been assumed to be associated with conditions imposing 'stress' on the animal. Various forms of 'stress' have resulted in a depletion of the stainable material some of which would be encountered in life and others which are purely artificial, e.g. subcutaneous formalin injection (Barrnett, 1954). Using Bargmann's modification of Gomori's chrome-alum haematoxylin method (Gomori, 1941) or the aldehyde fuchsin method of Halmi (1952), depletion of the neurosecretory material can be demonstrated following such stimuli, though there is still some doubt whether these methods stain the hormones themselves or merely a carrier substance (Barrnett, 1954; Sloper, 1955).

That the posterior pituitary does respond to brief periods of severe stress of various types, as shown by a depletion of the Gomori-positive material, has been reported by Barrnett (1954), Rothballer (1953, 1956) and Scharrer & Frandson (1954). Rennels & Russell (1957), however, could find no such depletion, nor decrease in the posterior pituitary hormones, using electrical stimuli of 10 sec. duration. There is evidence that the exposure of animals to heat invokes a secretory response in the posterior pituitary under long term (Itoh, 1954) or more acute and severe conditions (Robinson & Macfarlane, 1956).

The purpose of the following experiments was to investigate by histological methods whether this pituitary response could be demonstrated in mice reared in the heat or in those subjected to severe heat stress for short periods. Since in the acute experiments the animals were subjected to a lethal form of heat exposure, it became necessary to decide how far a non-specific 'stress' or 'shock', as distinct from the osmotic stimulus of dehydration, might be involved. Subsidiary experiments in which the animals were exposed to chronic dehydration or to cold stress were carried out in an attempt to make this distinction.

METHODS

Ninety-eight mice from the genetical strain C57 and A, as well as hybrids between them, were used in the experiments. After being reared for 3 weeks at room temperature (21° C. dry-bulb, 12° C. wet-bulb) the litters were separated after weaning into two groups similar in respect of sex and weight. One group, the 'cool-reared', remained in this environment while the other, the 'heat-reared', was transferred to a heated chamber maintained at 33° C. dry-bulb, 29·5° C. wet-bulb. They otherwise received identical treatment as regards food, accommodation and illumination. All animals were 8 weeks old when used for the experiments.

A. Main investigation

For the heat-shock experiments the animals were weighed and then transferred simultaneously from both environments to an automatically controlled chamber at a temperature of 41·7° C. dry bulb-, 29·4° C wet bulb (relative humidity 40 %), except for representatives of each litter from both heat and control chambers which were retained as non-shocked controls. Four such heat-shocks were carried out using forty-four mice with sixteen unexposed controls. Throughout each experiment the mice were supplied *ad lib.* with food and water. Individuals were then removed at hourly intervals up to 12 hr., weighed and decapitated. Any others succumbing during that period were also weighed as soon as possible after death; there were nine such animals out of the forty-four exposed. In addition, twenty more animals which did not survive the exposure to heat and eighteen control animals were available for study from the series of experiments carried out by Dr G. A. Harrison (1957, 1958) in connexion with his work on heat adaptation and tolerance in mice. Though not referred to in detail in the text, they confirm the findings on the animals reported here. Two mice in one of the heat-shocks surviving the entire length of the experiments were not decapitated until the following morning having spent the intervening hours at room temperature.

In an attempt to alter the severity of the experimental conditions, two litters of mice (12 animals) were subjected to a still higher temperature and greater relative humidity (46·1° C. dry-bulb, 38·9° C. wet-bulb, relative humidity 60 %). Two mice were removed after 14 min exposure, the remainder succumbed after 30–35 min. The conditions of the experiment other than the temperature were the same as in the previous ones.

B. Subsidiary experiments

For the experiments on saline dehydration the procedure of Leveque & Scharrer (1953) was repeated using 3 % saline. Six mice, two cool-reared and four heat-reared, were given this solution as drinking water, but were otherwise maintained on a normal diet in the environment to which they were accustomed. One of these mice was decapitated on the second day of the experiment and another on the seventh day; the remainder were killed after 15 days.

A second experiment was then conducted in which ten mice from two litters, all cool-reared, were given a normal diet but with 6 % saline instead of drinking water. These were killed at 24-hourly intervals beginning after 3 days until the seventh day when the remainder were decapitated.

In the cold stress experiments, ten animals from two litters were exposed to a temperature of −8° C (±2° C.) for up to 4 hr.; of these, two mice which survived were kept alive until the following morning at room temperature.

C. Treatment of the material

As soon as possible after decapitation (2–3 min. at the most), the cranium was removed to expose the brain and attached pituitary, and the whole head immersed in Bouin's fluid for 2 hr. The partially hardened tissues were then dissected from the skull and left for a further 22 hr. in the fixative. Serial sagittal sections of the hypothalamus and attached pituitary were cut at 5μ. From these sections, three series were mounted and stained respectively, under standard conditions, with aldehyde fuchsin, Bargmann's modification of Gomori's chrome-alum haematoxylin and by Masson's trichrome method. Of these, the first two were used to detect the presence of neurosecretory material, while the Masson-stained slides were used for studying the general cytology and for measuring the size of the pituicyte nuclei. For the latter purpose the two sections having the greatest longitudinal diameter of each posterior pituitary, were projected horizontally on to paper at a magnification of ×1270 and the areas of the pituicyte nuclei traced. At this magnification they were sufficiently sharp in outline to avoid significant errors in tracing, and yet large enough to be later measured with a planimeter. An average nuclear area was calculated from approximately fifty readings for each animal, and expressed in sq. mm. at this magnification.

RESULTS

A. Heat-shock experiments

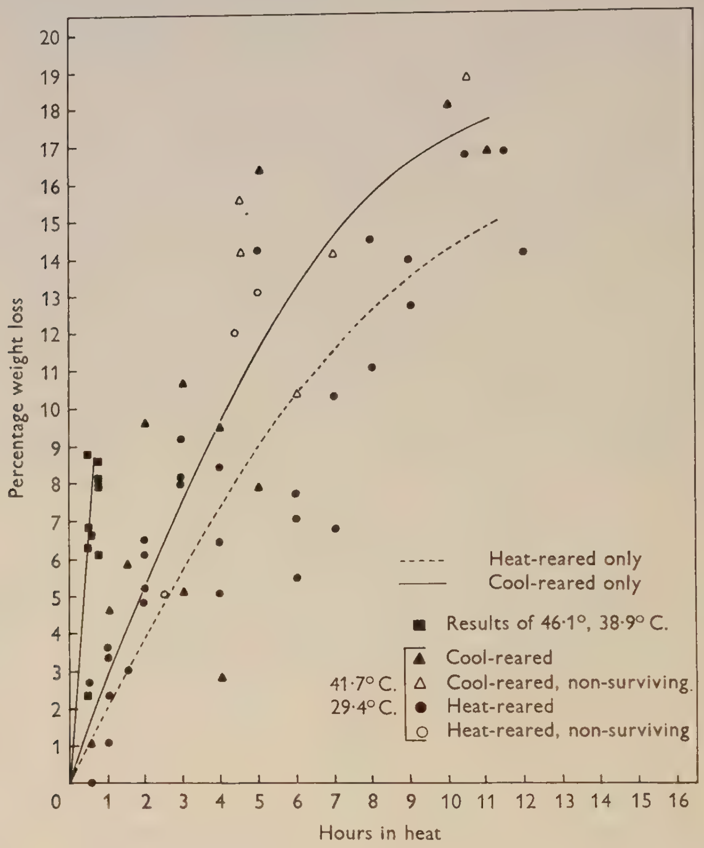
Survival times of the mice, calculated from these and other preliminary experiments, were generally from 8–12 hr. for the hybrid animals and rather less for the inbreds of either genetical strain. Though the animals were supplied *ad lib.* with food and water during the experiments, they rarely ate and were only seen to drink consistently during the first few hours of exposure to heat.

In accordance with the results of Harrison (1957), the heat-reared animals tended to have a slower rate of dehydration than the cool-reared, though the curves illustrating the difference between the weight losses of the heat-reared and cool-reared animals (Text-fig. 1) are not quite significant at the 5% level with the limited number of animals used here. Comparison of the curves derived from the dehydration rates of the surviving animals, with the data obtained from the non-surviving animals, indicates that the latter were tending to dehydrate more rapidly (Text-fig. 1).

At the higher heat-shock temperatures these differences in weight loss between the heat- and cool-reared animals are not apparent, as all the mice lost weight rapidly. During the lethal exposure period of 30–40 min they lost as much as 5–8% of their initial weight as compared with a 1–2% loss of comparable animals during the same period in the less severe climate.

Pars nervosa

(i) *Gomori-stained sections.* Microscopically there is a striking difference between neurohypophyseal sections obtained from control animals (i.e. those not subjected to the heat-shock) and sections obtained from animals succumbing during exposure. The typical picture presented by the former, when stained with chrome-alum haematoxylin (Pl. 1, fig 1; Pl. 2, fig. 3) is similar to that described by many previous workers. Irrespective of whether the animal has been heat- or cool-reared (Pl. 1, Fig. 1, 2), the picture consists of a more or less uniform distribution of small Gomori-positive granules completely obscuring any other cellular details such as nuclei and blood vessels. The density of the stained material may vary quite considerably from one control animal to the next, but the uniformity of staining is characteristic. In sections of the posterior pituitary obtained from animals which succumb to the heat however, the granules are by no means so homogeneously distributed (Pl. 2, fig. 4). They appear to form much larger aggregations possibly due to coalescence and their distribution is less uniform as they tend to collect round the distended blood vessels, outlining them against the vacuolated pituicyte cytoplasm. This redistribution is characteristic of these animals, there was never merely a decrease in the density of the neurosecretory material, nor was a selection ever obtained devoid of Gomori-positive material. Mice reared in either environment and succumbing to the heat-shock showed a similar picture of reduction.



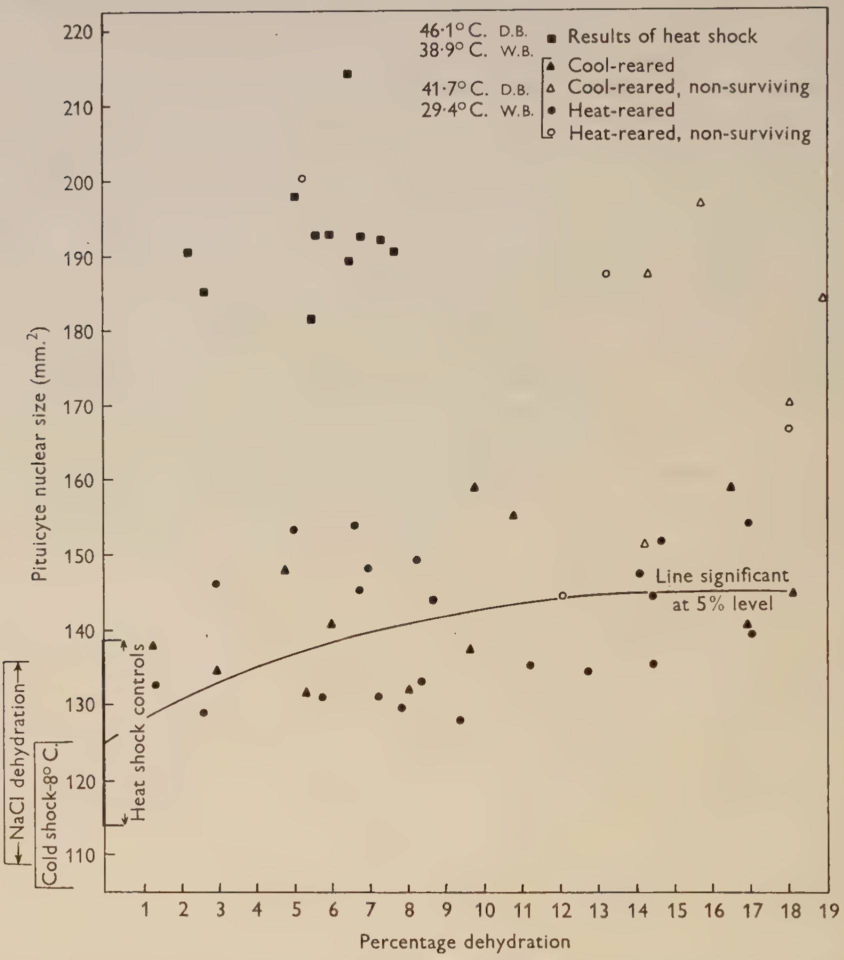
Text-fig. 1. Percentage weight loss of mice exposed to the heat shock experiments.

To determine at which stage in the experiment these changes occurred, animals removed at hourly intervals from the less acute experiment were examined. No differences could be detected in the Gomori-stained sections between animals which survived the heat-shock and the unexposed controls. It is difficult to reconcile these results with Rothballer's statement that a depletion of neurosecretory material can be seen after a moderate pain stimulus within an interval of only a few minutes. In our experiments, even when the animals surviving 10 hr. of 41·7° C. dry-bulb, 29·4° C. wet-bulb heat-stress had been dehydrated to the extent of losing 14 % of their original weight, there was still no definite change in the histological picture from that of the controls. There may possibly have been a decrease in the density of staining of the Gomori-positive material, but this could not be demonstrated conclusively either by simple microscopical investigation or by crude density measurements. Only if the animal had succumbed to the heat-shock or was moribund at the time of decapitation, was it possible to distinguish definitely between the control and the experimental slides, by the size of the nuclei, the distension of the blood vessels and the vacuolation of the pituicyte cytoplasm. In the two mice surviving the whole period of the heat-shock and allowed a 12 hr. recovery period at room temperature, the microscopical sections obtained suggest that the Gomori-positive material in the neurohypophysis was greater in amount than even the most dense of the control range. There was also no vascular distention.

The results obtained from the twelve animals subjected to the more severe heat-shock confirmed those already described. Slides from animals removed after 15 min. exposure could not be distinguished from the controls, while sections obtained from their litter-mates succumbing to the heat stress 15 min. later, again presented a typical, but less dramatic picture of depletion, not so readily distinguishable by low-power investigation from the non-exposed controls as comparable slides from the less severe experiment. The blood-vascular distension in particular was very varied and ranged from negligible to extreme.

(ii) *Masson-stained sections.* These slides revealed further cytological differences between unexposed controls (Pl. 3, fig. 5) and non-surviving animals (Pl. 3, fig. 6). In the latter series it could be seen that the nuclei of the pituicytes were considerably larger and stood out more conspicuously in their vacuolated cytoplasm; the chromatin material was concentrated along the nuclear membrane while the central part of the nucleus appeared vacuolated and unstained. The nucleolus was more darkly stained than normal. A further distinguishing feature was that these nuclei tended to be more rounded in outline. Planimeter measurements showed that their average area, in all but two of the non-surviving animals, was about 50 % greater than that of the control series. The reason for the two exceptional cases is not apparent. Comparable tracings taken from nuclei in the hypothalamus of the same mice showed that this nuclear enlargement was localized in the pars nervosa and not due, therefore, to a generalized effect of the experimental conditions on brain cells.

On averaging the pituicyte nuclear areas for each control and heat-shocked animal and plotting these values against percentage weight loss, the resulting curve, omitting the results from the non-surviving animals, revealed a trend just significant at the 5 % level (Text-fig. 2). This curvilinear relationship shows that, after an appreciable increase in the size of the pituicyte nuclei for the initial 10–12 % weight loss, further dehydration had no significant effect unless the animals died. An impression of a greater nuclear size in the cool-reared animals was not supported by statistical examination. The results for the animals which succumbed to the heat were then superimposed, and as can be seen from the figure (Text-fig. 2) the sizes



Text-fig. 2. Pituicyte nuclear sizes of surviving and non-surviving mice exposed to the heat shock experiments. Each point represents an average of fifty readings. The curve is based on readings from surviving animals only. The range of pituicyte areas from the subsidiary experiments are indicated on the ordinate at the left.

were considerably larger. In this series there was also some tendency for the animals which died earlier at a lower percentage dehydration to show larger nuclei and more distended blood vessels, than those dying later in the experiment. This was confirmed by the heat shocks performed at the higher temperature, where the temperature tolerance of the mice was much less and there were no survivors after 30 min.; even though these animals never achieved more than 8 % dehydration, their pituicyte nuclear sizes were generally larger than those obtained from the previous experiment at the lower temperature (Text-fig. 2).

The hypothalamus. Observations were also carried out on the paraventricular and supraoptic nuclei of the hypothalamus. Owing, however, to the great variability in the amount of Gomori-positive material in these nuclei of even the cool-reared control animals, it was found impossible to determine for certain any change either in the heat-reared controls or in the heat-shocked animals. The amount of staining in the non-surviving mice did not differ from that of the other experimental groups of animals. It would appear that the reduction in the Gomori-stained material in the neurohypophysis, characteristic of animals succumbing to the heat, is not reflected in a similar depletion in the hypothalamus.

B. Subsidiary experiments

(1) *Sodium chloride dehydration*

The purpose of these experiments was to investigate whether the changes in the neurohypophysis characteristic of the non-surviving mice, particularly the enlarged nuclei, could be attributed entirely to the effects of dehydration.

The six animals given 3 % sodium chloride to drink lost 20 % of their weight in the first 48 hr. of the experiment. They then appeared to adapt, at least temporarily, to this diet, for in the following 9 days they lost on the average only another 10 % of their body weight.

Histologically there was very little change in the neurohypophysis for the first 4 days, in spite of the loss of body weight. There was a gradual reduction of the Gomori-positive material but no reorganization, characteristic of the mice which did not survive the heat shock experiment, even though they suffered a comparable degree of dehydration. By the 15th day there was an almost total depletion of the stainable material, but in the Masson-stained slides the sections from experimental animals were still indistinguishable from the controls. There were no distended blood vessels, the nuclei appeared normal and the cytoplasm was unvacuolated.

The ten more drastically treated mice, supplied with 6 % saline solution showed a serious deterioration in their condition within 7 days after a linear weight loss of 35–40 % of their body weight. The sections prepared from these animals showed a total loss of Gomori-positive material within 4 days, at which time the pituicyte cytoplasm was becoming vacuolated and the entire posterior lobe shrinking in size. The pituicyte nuclei appeared small and pyknotic. With the total disappearance of the stainable material from the posterior lobe there appeared to be a reduction in the neurosecretory content of the hypothalamic nuclei, leaving, at death, only a few palely staining Herring bodies in the median eminence, though, owing to the variability in the amount of the stainable material in the hypothalamus of a control animal, this was difficult to prove.

With one exception, in none of the slides examined of the saline-treated animals, was there a distension of the capillary system in the posterior lobe comparable with that seen in the non-surviving mice from the heat shock. This animal died during the night and consequently the tissues were not fixed until at least 2 hr.

post-mortem; in these sections alone was there a dramatic distension of the blood vascular tissue in the region. The histological appearance of the neurohypophysis of the non-surviving mice from the heat shock experiment is not reproduced by either slow or rapid sodium chloride dehydration.

(2) *Cold stress experiments*

These experiments were planned in order to see whether a form of acute stress, other than that of severe heat, would produce similar effects on the neurohypophysis.

The animals exposed to the cold survived for between 50 min and 4 hr. Those reared at room temperature (the cool-reared) showed a greater tolerance to the conditions of the experiment than the heat-reared, and the hybrids a greater tolerance than the inbreds. The sections of neurohypophysis obtained from the non-surviving animals, however, could not be distinguished from those of normal controls, nor from the surviving animals which were killed the following morning after 4 hr. of exposure to the cold followed by 16 hr. of recovery at room temperature. There was no depletion of the Gomori-positive material, no vascular distension and no hypertrophy of the pituicyte nuclei (Text-fig. 2). No changes, either, could be found in the hypothalamic nuclei.

DISCUSSION

By using solely histological techniques, it was not possible to find any differences in the neurohypophysis between animals reared for 8 weeks in moderate heat (33° C. dry-bulb, 29° C. wet-bulb) and litter-mates reared at room temperature. However there must have been physiological adaptation as the heat-reared animals survived better in the conditions of the less severe heat shock (Harrison, 1958). Although the pituicyte sectional area may perhaps be taken as an indication of activity in the posterior pituitary, there was no significant difference in this between the two sets of animals.

The results of the experiments described indicate that the cytological responses of the posterior pituitary to dehydration, as imposed by the acute heat-shock and by chronic administration of a hypertonic saline solution, are different. A rapid dehydration, such as produced by the heat-shock experiment, resulting in a weight loss of 15 % and eventually causing the death of the animal, induced a considerably greater response than a slow dehydration of the same degree and to which the animal had appeared, at least temporarily, to become adapted. These results also show that the neurohypophyseal response to 'stress', as applied during these experiments, was not nearly as marked as was expected from previous reports (Rothballer, 1953; Scharrer & Frandson, 1954; Barrnett, 1954).

In the rapid dehydration produced by the lethal temperatures, pronounced liberation of the antidiuretic hormone might have been expected, though this could not be detected by observations on the stained sections. Physiological experiments to demonstrate the level of hormone in the posterior pituitary would probably have shown this, as it would seem reasonable to suppose from the significant increase in the size of the pituicyte nuclei during this phase that activity of some sort was taking place in the neurohypophysis. Tala (1953), for instance, has shown that nuclear volumes in the follicle cells of the thyroid are a direct indication of the activity of the gland. The visible depletion when it did occur, only at the death of the animal, was of quite a different type to the gradual disappearance characteristic of the sodium chloride dehydrated mice. The typical clumping and reorganization of the neurosecretory material was never seen in the latter experiment and at the moment no explanation can be offered as to its significance. However, it seems clear that the changes seen in the mice which did not survive the heat-shock were not due to the absolute dehydration which they had undergone. Nor were they due to the 'stress' of the experiment, as the severe conditions imposed by the cold had no apparent effect, either on the pituicytes or on the Gomori-positive material.

Sections obtained from the animals subjected to the severe heat-shock (46·1° C. dry-bulb, 38·9° C. wet bulb) do, however, have features in common with those exposed to, and not surviving, the less severe experiment. Though there was a less detectable difference between Gomori-stained sections obtained from control and non-surviving animals, in the former experiment due to the smaller degree of vacuolation of the pituicyte cytoplasm, high-power microscopical investigation invariably revealed enlarged nuclei. The Masson-stained slides were unmistakably identifiable owing to the size of the nuclei and the distended capillaries. This latter feature is often obscured, unless very marked, in the sections stained to show neurosecretory material.

The part played by the pituicytes in the storage and release of the neurohypophyseal hormones is still not clear. It is thought that they are active, instead of being mere supporting cells (Rennels & Drager, 1955), but the manner in which they effect the transference of the secretory material from the nerve endings to the lumen of the capillaries is still unknown. A few previous workers have commented on the change in size of the pituicytes, or their nuclei, under normal or experimental conditions. In 1951 Ortmann reported the existence of larger nuclei in the pars nervosa of thirsting rats, though this was contrary to the reports of Chambers (1945) and more recently Rennels (1958) who stated that he saw no differences in the pituicytes of rats similarly treated. Certainly there was no hypertrophy in thirsting mice in our experiments, where the nuclei, if there was any change, were pyknotic. Collin & Racadot (1953) and Stutinsky (1953) mentioned an increase in the size of the pituicyte nuclei, also linked with distension of the blood capillaries in the neurohypophysis of guinea-pigs and rats during parturition and gestation; though more recently Racadot (1957) has thrown some doubt on this statement. Rennels (1958) reported hypertrophied pituicytes which were invariably found in multiparous females. He attributes this to repeated demands upon the release of the oxytocic hormone during lactation, though he was unable to produce the phenomenon experimentally by artificially prolonging the period of lactation. Hypertrophy of the cytoplasm, nucleus and nucleolus of the neurones of the supraoptic and paraventricular nuclei of the hypothalamus have been reported by Kovacs, Bachrach, Jakobovitz, Horvath & Korpassy (1954) following thirsting in rats. This they also take as an indication of increased secretory activity.

From our experiments it would seem that pituicyte nuclear hypertrophy occurs only in response to acute experimental procedures known to involve the rapid release of the antidiuretic hormone. Severe chronic demands do not result in a similar response. This would support the view that the pituicytes play an active part in the release of the antidiuretic hormone, but throws no light on the method by which this is brought about.

In spite of the distension of the blood vessels in the pars nervosa of the non-surviving animals, stainable material was never found within them. Whether this distension was an active one induced by a rapid release of the neurosecretory material, or whether it was a passive relaxation of the walls of the blood vessels at death, is not known. Distension was never noticed in the heat-shocked animals unless they were dead or moribund, and the only sodium chloride treated animal to show this feature was the one which had been dead for some hours before the tissues were fixed. However, mice succumbing to the cold shock showed no such distension. The fact that other workers, notably Scharrer & Frandson (1954) and Rothballer (1953, 1956) (using pain stimuli) and Collin & Racadot (1953) (employing normal physiological stimuli) have also reported this phenomenon in animals decapitated while living, would suggest that this is a natural active process, probably stimulated by an acute demand for either of the two posterior pituitary hormones.

It appears that histological changes characteristic of lethal heat exposure can be produced in the neurohypophysis, which are different to those found following other forms of stress. They confirm that the neurohypophysis is involved in the response to heat stress, but they do not help to decide whether the hypothalamus is similarly involved.

Since the completion of this paper an additional publication has been brought to my attention [‘Gomori substance in the neurohypophysis of rats exposed to heat and cold’, Ueno T. (1957), *Nagaya J. Med. Sci.* **20**, 75]. While Ueno exposed winter and summer rats for only 120 min. to a severe hot climate (41° C. dry-bulb, 34° C. wet-bulb) he describes essentially the same histological changes in the posterior pituitary as those detailed in the present paper. These include oedematous cytoplasm, the development of vacuoles round the swollen pituicyte nuclei and hyperaemia and vasodilatation as well as the reorientation of the Gomori positive material. While this confirms the observations described in the present paper it differs from them in that the stress alone was sufficient to produce these changes; he makes no reference to any experimental animals succumbing to the conditions. Yet it was a consistent feature of our experiments that histologically, surviving animals could not be distinguished from the controls.

No explanation for this difference has yet been suggested.

SUMMARY

1. No difference could be found either in neurosecretory content or in general cytology in the pars nervosa, between animals reared in a warm climate and those reared at room temperature.

2. The pars nervosa of mice reared in either environment and subjected to a lethal temperature showed characteristic changes at death, including vacuolated pituicyte cytoplasm, distended blood vessels and enlarged pituicyte nuclei. No stained neurosecretory material was ever seen in the lumen of the capillaries.

3. These changes were not produced by lethal dehydration by sodium chloride solution, nor by lethal cold exposure. The neurohypophyseal response to 'stress' as imposed by these experiments was not as marked as had been expected.

4. The significance of these observations is discussed in relation to the release of the neurohypophyseal hormones.

I am greatly indebted to Sir Wilfrid Le Gros Clark, F.R.S., and Dr J. S. Weiner for their help and encouragement throughout the preparation of this paper, and to Dr A. Howe for his criticism of the manuscript. I should also like to thank Mr C. W. Graham and Mr B. Purvis for their technical assistance and Mr F. Blackwell for the preparation of the photomicrographs.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Sagittal section through pituitary of a cool-reared control animal. Note uniformity of staining. Chrome-alum haematoxylin technique, × 235.

Fig. 2. Sagittal section through pituitary of a heat-reared control animal. Note similarity to fig. 1. Chrome-alum haematoxylin stained, × 235.

Plate 2

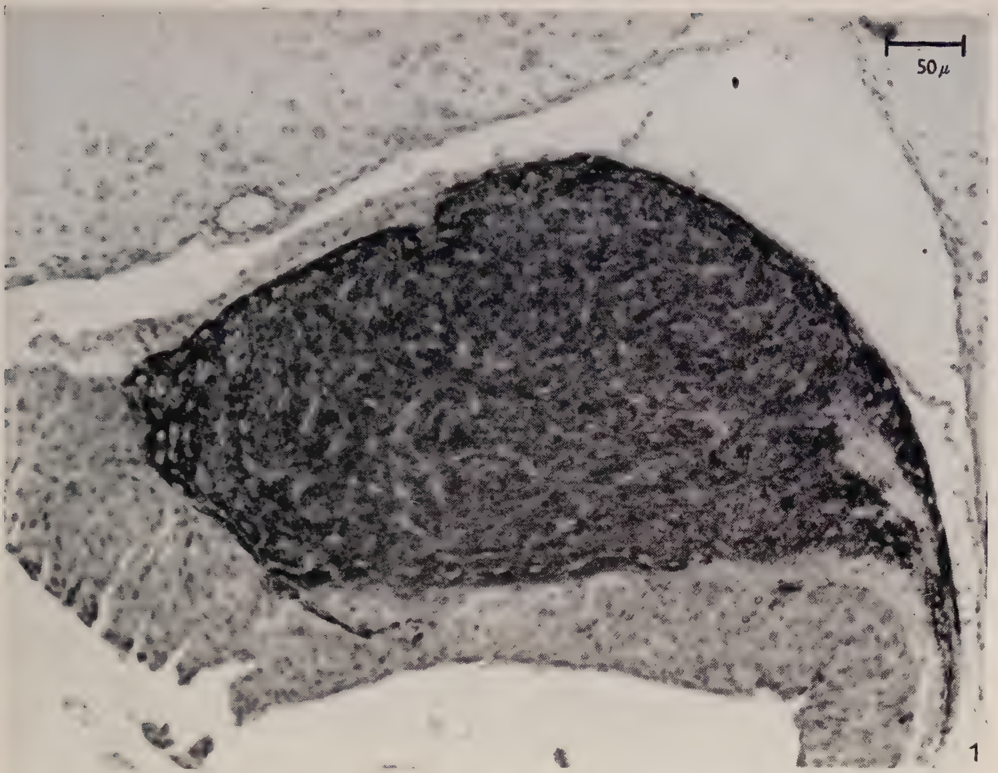
Fig. 3. Sagittal section through pars nervosa of a cool-reared control animal showing lack of any cytological details due to the presence of the neurosecretory material. Chrome-alum haematoxylin stained, × 850.

Fig. 4. Pars nervosa of a mouse which succumbed to the heat stress. Note the vacuolation of the pituicyte cytoplasm and the appearance of enlarged nuclei, also the 'coalescence' of the neurosecretory material. Chrome-alum haemotoxylin stained, × 850.

Plate 3

Fig. 5. Adjacent section to fig. 3 stained with Masson's trichrome stain, × 850.

Fig. 6. Adjacent section to fig. 4 stained with Masson's trichrome stain. Note the greatly enlarged pituicyte nuclei, vacuolated cytoplasm and distended blood vessels, × 850.



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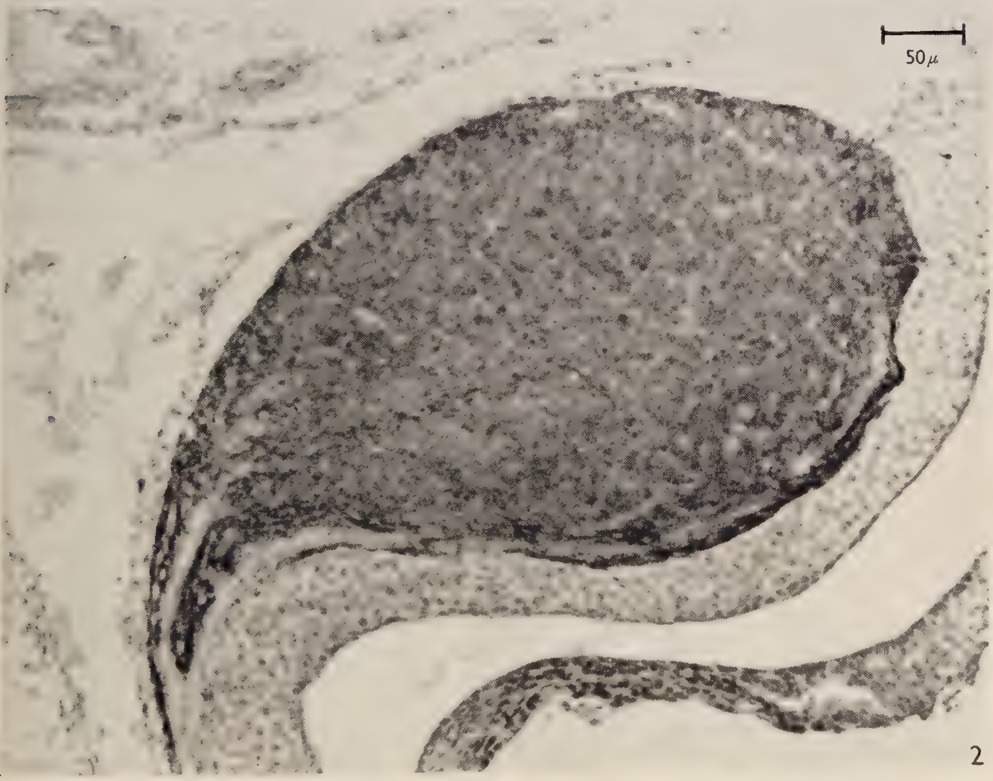
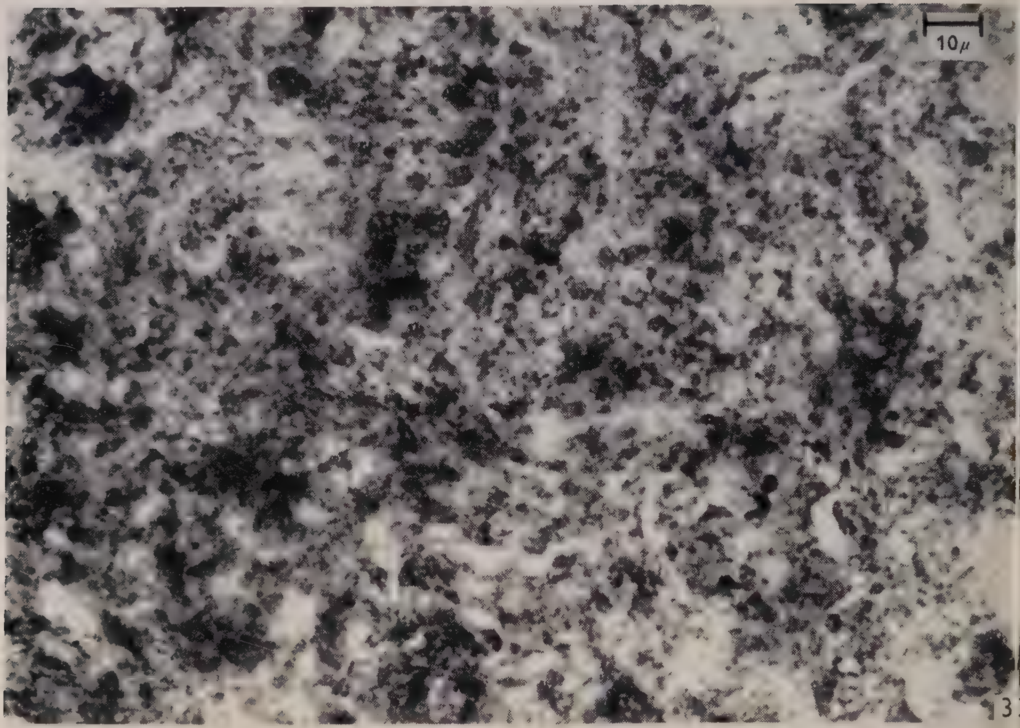


2

CULLINGHAM—The influence of heat-stress on the pars nervosa of the mouse

(Facing p. 374)



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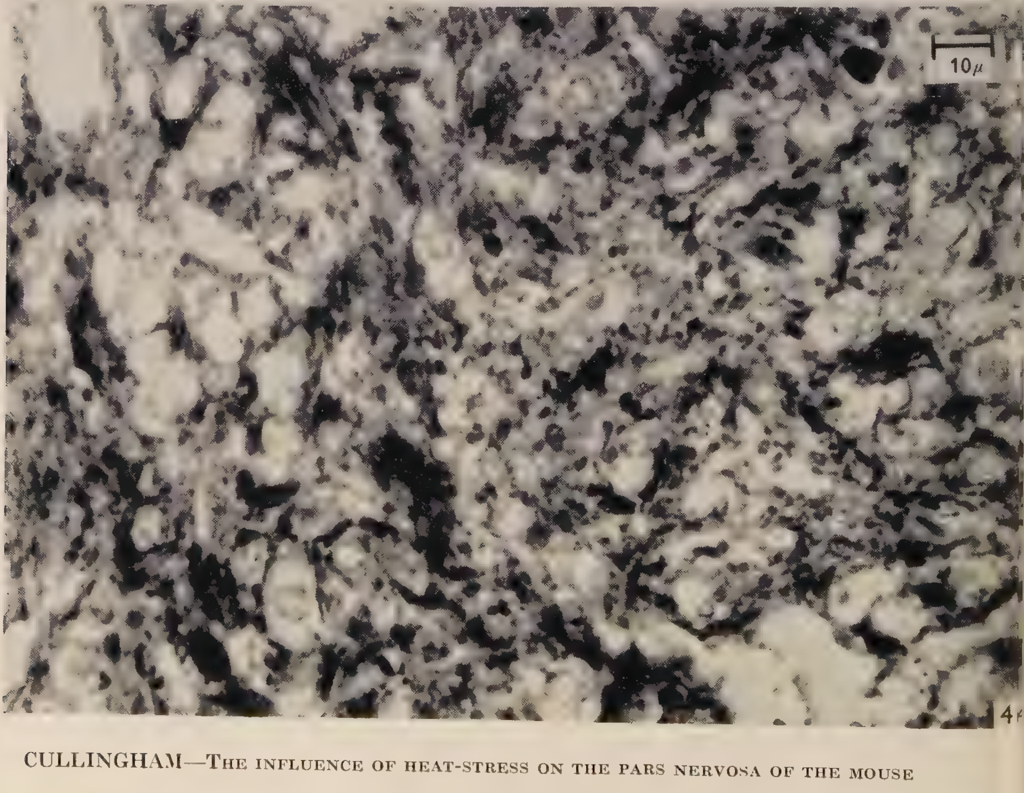

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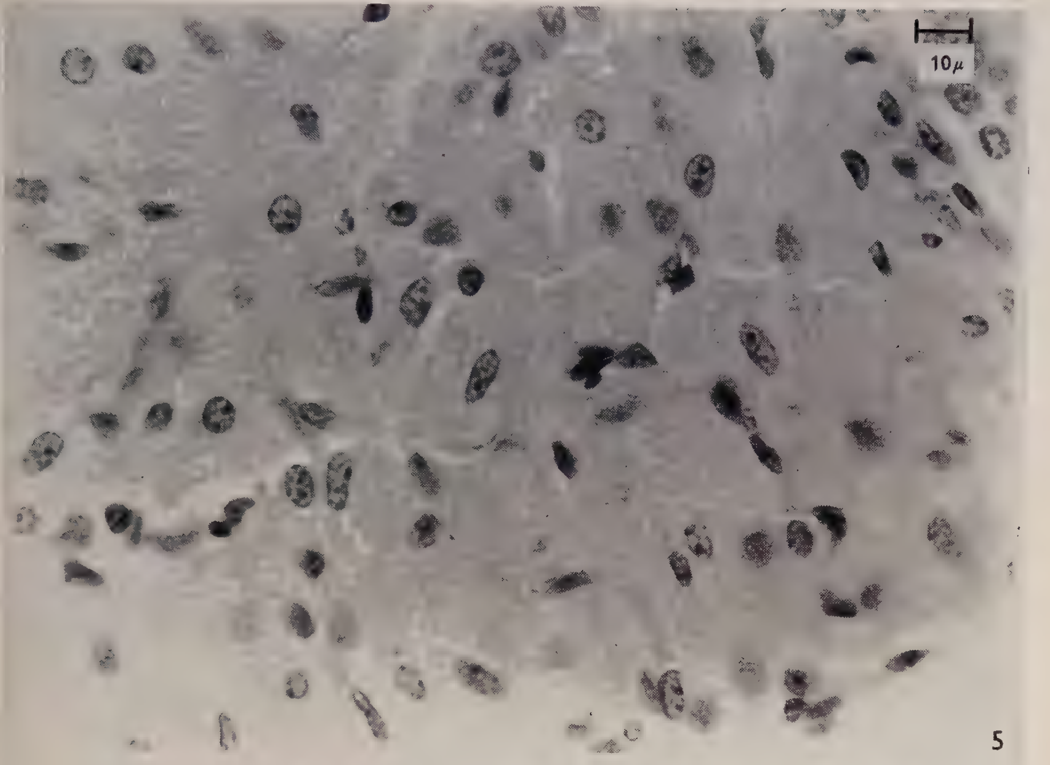
CULLINGHAM—THE INFLUENCE OF HEAT-STRESS ON THE PARS NERVOSA OF THE MOUSE



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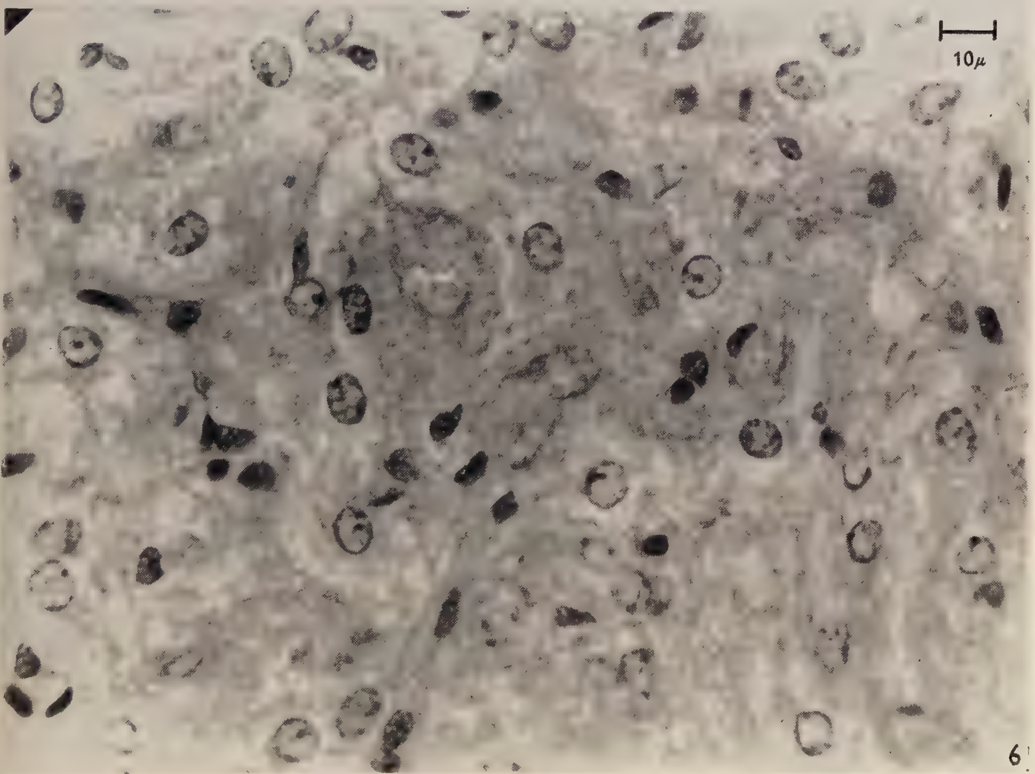

6

CULLINGHAM—The influence of heat-stress on the pars nervosa of the mouse

EXPERIMENTAL AND HISTOLOGICAL STUDIES OF THE EXTRAPETROUS PORTION OF THE FACIAL NERVE AND ITS COMMUNICATIONS WITH THE TRIGEMINAL NERVE IN THE RABBIT

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INTRODUCTION

Spindles and other types of sensory ending have been found in the facial muscles of man (Kadanoff, 1956) and in the m. quadratus labii superioris of the rabbit (Bowden & Mahran, 1956), but as yet the source of their nerve supply is unknown. Before these endings had been demonstrated, there were three conflicting views about the afferent innervation of mimetic muscles: its existence was denied; some others considered that it was derived from the facial nerve and the third view was that it was from the trigeminal (Mahran, 1955). If the latter hypothesis were true, the peripheral communications between the trigeminal and facial nerves might provide one path by which sensory fibres could travel centrally from the muscles. On the other hand, Hirschfeld & Léveillé (1866), Hovelacque (1927), Huber (1930) and O'Connell (1936) considered that although communications between the two nerves existed, there was no intermingling of their fibres. Indeed, the two latter authors stated that the facial nerve only served to distribute the trigeminal fibres to the skin and its appendages, and possibly to blood vessels. However, no histological examination appears to have been made, and the present study in the rabbit was designed to count and measure the fibres of the facial nerve below the stylomastoid foramen, to trace the course of the fibres of the trigeminal which communicated with the facial nerve and lastly to determine the number and size of any trigeminal fibres which might enter the m. quadratus labii superioris. The topographical anatomy of the extrapetrous communications between the two cranial nerves is illustrated in text-fig. 1 (based on thirty-two dissections which form part of another study which is in preparation).

MATERIAL AND METHODS

Eleven healthy adult rabbits were used in this experiment. The animals were divided into four groups. The first group consisted of two healthy intact animals. In the remaining nine animals the right facial nerve was divided below the stylomastoid foramen under ether anaesthesia with full aseptic precautions. The animals were killed at periods ranging from 7 to 53 days after operation. Full details of the operative technique have been given by Mahran (1955). In order to preserve the communications between the facial nerve and the auriculo-temporal nerve, the trunk of the facial nerve was mobilized by dividing the posterior auricular nerve and the facial nerve was cut immediately below the foramen. To prevent regeneration of fibres into the distal end, both proximal and distal stumps were treated with 1% gentian violet (Guttmann & Medawar, 1942). The distal stump was then turned back on itself and sutured to surrounding tissues. Animals 3–6 were placed in group II, 7–10 in group III and number 11 was the sole member of group IV.

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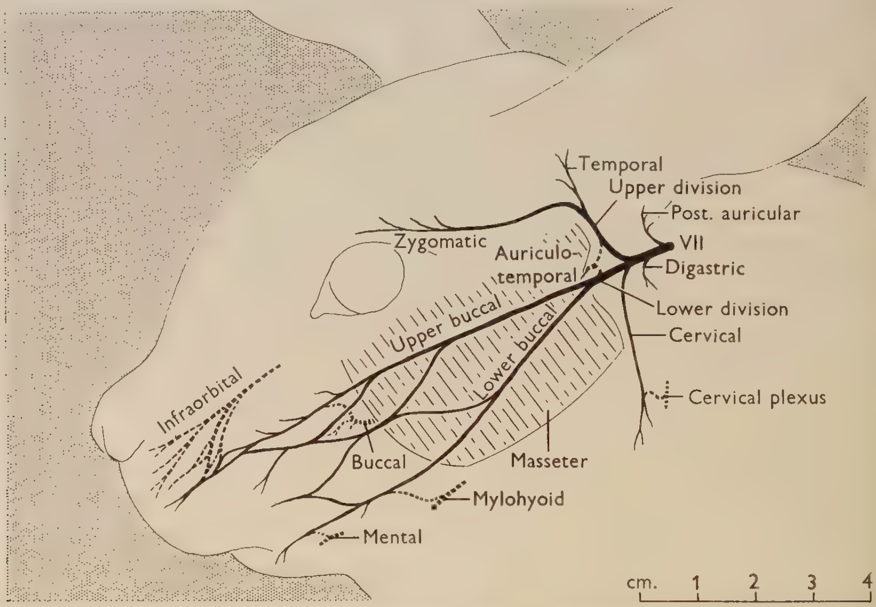
* Present address: Department of Anatomy, Khartoum University. The early work in this investigation was presented as part of a Thesis on 'Innervation of Facial Musculature' for which the degree of Ph.D. was awarded by the University of London in 1955. The greater part was completed in 1959, whilst in receipt of a personal Grant from the Wellcome Foundation.

Animals in group II were given an overdose of ether and were immediately perfused with normal saline followed by 10% formol saline. The tissues studied were embedded in paraffin, serial sections were cut and stained either by the Holmes (1942) or Romanes (1950) silver techniques. Details of treatment and the histological procedures are given in Table 1.



Text-fig. 1. The facial nerve and its communications with the trigeminal in the face of the rabbit.

In groups I, III and IV, the tissues were dissected out from fully anaesthetized animals which were later killed with an overdose of ether. The nerves from animals in group III were placed on a piece of filter-paper and left in 1% osmic acid for 24–36 hrs.; further treatment of these tissues is given in Table 2. In groups I and IV the nerves were fixed and stained with osmic acid, embedded in paraffin and sectioned serially at 5 μ. The fibres were measured from photographs of transverse sections enlarged 750 times in the manner described by Fernand & Young (1951). Mosaic photographs were constructed for the larger specimens (e.g. Pl. 1, fig. 1; Pl. 2, figs. 6, 7; Pl. 3, fig. 8). In the early stages of the work, prints were made on bromide and on non-shrink paper, but since comparison of the results showed negligible differences, bromide paper only was used in the later stages as it was more economical and easier to handle in constructing the mosaics, the largest of which measured 5 ft. × 2 ft.

Table 1. *Animals in group II*

Animal serial no.	Post-op. survival period (days)	Material from operated side	Methods	
			Sectioning and mounting	Stain
3	7	VII and com. with V (A.T., B. and I.O.) from site of operation to M.Q.L.S.	*15 μ. Every section	Holmes
		VII and com. with V (M.H.)	*15 μ. Every 10th pair	
		M.Q.L.S.	L.S. 15 μ. Every 5th section	Romanes
4	14	VII and com. with V (A.T.) from site of operation to ant. border of masseter VII and com. with V (M.H.)	*15 μ. Every 5th pair	Holmes
		Two branches to m. buccinator	T.S. 15 μ. Every section	Holmes
		M.Q.L.S.	L.S. 15 μ. Every 5th section	Romanes
5	21	VII and com. with V (A.T. and B.) from site of operation to M.Q.L.S.	*15 μ. Every 5th pair	Holmes
		VII and com. with V (I.O.)	L.S. 10 μ. Every section	
		Two branches to M.Q.L.S.	T.S. 15 μ. Every 5th pair	
		M.Q.L.S.	L.S. 15 μ. Every 5th section	Romanes
6	31	Five branches to m. buccinator	T.S. 15 μ. Every 10th pair	Holmes
		M.Q.L.S.	T.S. 15 μ. Every 5th section	Romanes

Table 2. *Animals in group III*

Animal	Survival period (days)	Material from operated side	Methods
7	53	5 branches to m. buccinator	Paraffin T.S. 15 μ. Every 10th pair
8	14	VII and com. with V (B. and I.O.)	Paraffin L.S. 10 μ. Every section
		VII and com. with V (M.H. and A.T.)	Teased in glycerine
9	21	VII and com. with V (A.T., M.H. and I.O.)	Teased in glycerine
10	14	VII and com. with V (A.T., B. and I.O.) from site of operation to M.Q.L.S.	Paraffin* 15 μ. Every 10th pair
		Three branches to m. buccinator	Teased in glycerine

Key to Tables 1 *and* 2

M.Q.L.S. = quadratus labii superioris muscle + skin and mucous membrane.
com. = communication.
A.T. = auriculotemporal nerve.
I.O. = infraorbital nerve.
B. = buccal nerve.
M.H. = mylohyoid nerve.

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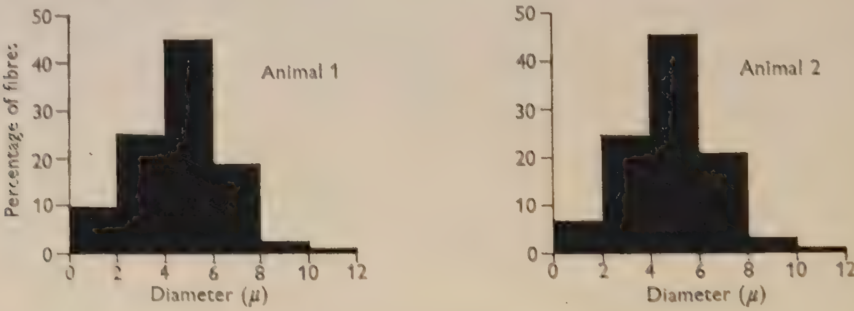
* The VIIth nerve was cut transversely and the communicating twigs of the Vth nerve were arranged in the paraffin blocks so that they were cut longitudinally.

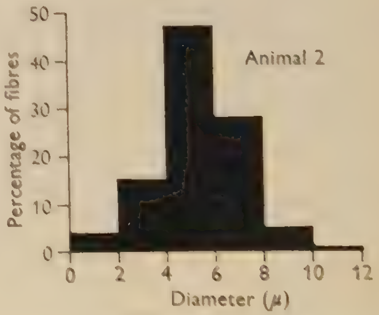
OBSERVATIONS

Normal nerves (group I)

(*a*) *Facial nerve trunk immediately below the stylomastoid foramen* (*Pl.* 1, *fig.* 1) *and the posterior auricular branch of the facial nerve.* The number of myelinated nerve fibres and their size-frequency distribution in nerves of the right side from animals nos. 1 and 2 are shown in Table 3 and Text-fig. 2. In each nerve the distribution is unimodal and there is a marked similarity in the histograms. The largest fibres measured 12 μ in diameter.



Facial nerve below stylomastoid foramen



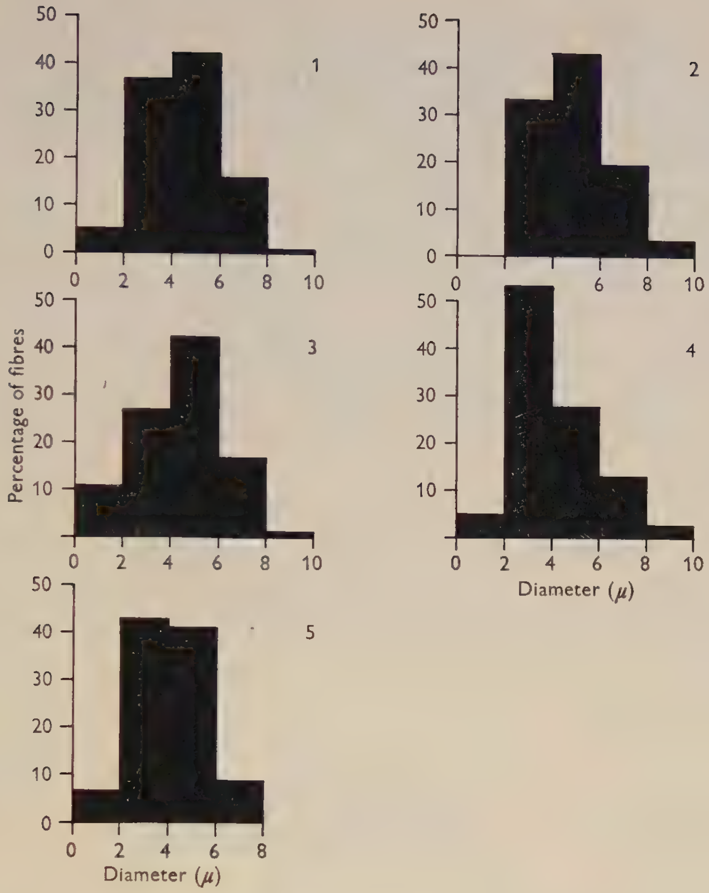
Post-auricular nerve

Text-fig. 2. Histograms representing diameter size-frequency distribution present in the normal facial nerve immediately below the stylomastoid foramen, and in the posterior auricular nerve immediately distal to its origin.

(*b*) *Vibrissal nerves.* Since fibres of the quadratus labii superioris muscle are inserted into the theca of the mystacial vibrissae and stout branches of the infraorbital nerve enter the muscle to supply them as well as other structures, five nerves from the largest vibrissae were examined in animal no. 2. The data are set out in Table 3 and Text-fig. 3. Measurements made 1–0·5 cm. proximal did not differ significantly from those taken immediately proximal to the theca and shown in Table 3. The measurements given for the fifth specimen were made within the sinus (Pl. 1, fig. 2) and the histogram shows a slight shift to the left.

Post-operative findings (groups II, III and IV)

A. Post-operative progress was uneventful and signs of facial paralysis were complete in all animals (Pl. 1, fig. 3). In animals nos. 3–11 inclusive, there was



Text-fig. 3. Histograms representing size-frequency distribution present in five vibrissal nerves in animal no. 2.

Table 3. *Fibre diameter size-frequency distribution in normal nerves of rabbit*

Animal serial no.	Nerve	Level of measurement	Numbers of myelinated fibres in each size group (μ)						
			0–2	2–4	4–6	6–8	8–10	10–12	Total
1	Facial rt.	Immediately below stylomastoid foramen	524	1330	2399	969	107	8	5337
		%	9·8	24·9	45	18·2	2·0	0·2	
2	Facial rt.	Immediately below stylomastoid foramen	414	1552	2922	1353	194	11	6446
		%	6·4	24·1	45·3	21·0	3·0	0·2	
2	Posterior auricular rt.	Immediately distal to point of origin	52	200	617	368	69	4	1310
		%	4	15·3	47·1	28·1	5·3	0·3	
2	Vibrissal N. 1	Immediately proximal to the theca	7	50	58	22	1	—	138
		%	5·1	36·2	42·0	15·9	0·7	—	
	Vibrissal N. 2		0	34	44	20	4	—	102
		%		33·3	43·1	19·6	3·9	—	
	Vibrissal N. 3		10	21	33	13	1	—	78
		%	12·8	26·9	42·3	16·7	1·3	—	
	Vibrissal N. 4		7	82	43	20	4	—	156
		%	4·5	52·7	27·6	12·8	2·6	—	
	Vibrissal N. 5	In sinus	12	75	71	15	—	—	173
		%	6·9	43·4	41·0	8·7	—	—	

evidence of complete degeneration of the facial nerve below the level of operation and no nerve fibres from any other sources had reached the cut end of the distal stumps. No intact nerve fibres were found until the first point of communication between the facial and trigeminal nerves.

B. *Communications between the facial and the mandibular division of the trigeminal nerve.* The gross anatomy of these communications is shown in Text-fig. 1. Histological study of serial sections and teased preparations (see Tables 2 and 3) showed that in every case the communications passed from the trigeminal into the facial nerve. There was no evidence of degenerating or degenerated fibres of the seventh nerve within these branches of the trigeminal which contained bundles of intact myelinated fibres. No attempt was made to determine the presence and the number of non-myelinated fibres in the main nerve trunks and communicating branches.

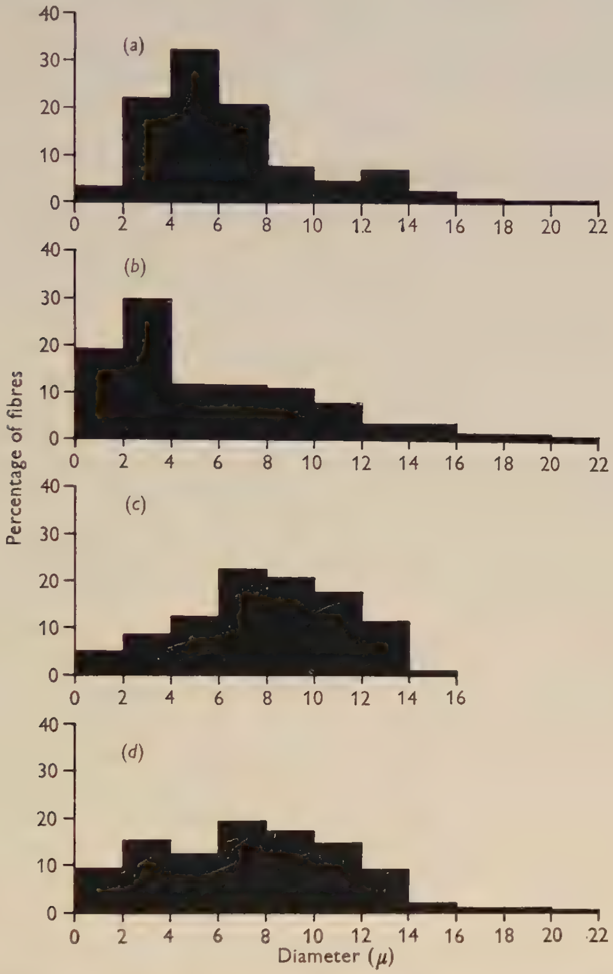
Table 4. *Fibre diameter size-frequency distribution for intact fibres of trigeminal nerve after communication with degenerated facial nerve in animal no.* 11, 28 *days after operation*

		Number of myelinated fibres in each size group (μ)										
Nerve	Level of measurement	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22
Upper buccal branch of VII	After intermingling of fibres from auriculotemporal nerve	29	175	253	165	57	36	54	17	4	2	2
	%	3·7	22	32	21	17·2	4·5	6·8	2·1	0·5	0·3	0·3
Upper buccal branches entering M.Q.L.S.	Immediately proximal to muscle	36	58	23	23	21	15	7	7	2	2	1
	%	18·5	29·7	11·8	11·8	10·8	7·7	3·6	3·6	1·0	1·0	0·5
		Intact infraorbital nerve fibres entering m. quadratus labii superioris										
Five bundles cut in T.S.	Immediately proximal to M.Q.L.S.	22	35	51	92	85	73	48	5	—	—	—
	%	5·4	8·5	12·4	22·4	20·7	17·8	11·7	1·2	—	—	—
		Intact fibres from infraorbital and auriculotemporal nerves entering M.Q.L.S.										
Combination of data given above		58	93	74	115	106	88	55	12	2	2	1
%		9·6	15·4	12·2	19	17·5	14·5	9·1	1·9	0·3	0·3	0·2

Percentage in Tables 3 and 4 is given as correct to the first decimal place.

When the intact fibres of the trigeminal nerve were traced distally, some were found to leave the combined seventh and fifth nerve trunk without further change. Other bundles remained in the combined trunk and broke up along its course. Bundles derived from the two nerves converged and broke up into smaller ones in which they intermingled. Isolated intact fibres were followed into otherwise denervated fasciculi and some of the intermingled fasciculi redivided into smaller mixed ones or into separate denervated and innervated bundles, which in some instances reunited (Pl. 1, figs. 4, 5) or joined other fasciculi to form larger mixed bundles (Pl. 2, fig. 6). In animal 11 the intact fibres were counted and measured in the upper buccal nerve (Table 4 and Text-fig. 4*a*). Seventy-nine fibres measuring over 12–22 μ in diameter were found distal to the auriculotemporal communication, these represented about a tenth of the population of intact fibres.

C. *Communications with the infraorbital nerve.* Many intact bundles of the infraorbital remained separated from the upper buccal branch of the facial nerve and supplied overlying skin, mucous membrane and vibrissae, but there was an intermingling of a proportion of fibres with the facial nerve and its contained contributions from the mandibular division of the trigeminal (Pl. 2, fig. 7; Pl. 3, figs. 8, 9).



Text-fig. 4. Histograms representing size-frequency distribution of intact trigeminal nerve fibres 28 days after section of the facial nerve immediately below the stylomastoid foramen. (*a*) In the upper buccal branch of the facial nerve distal to the communication from the auriculotemporal nerve. (*b*) In the upper buccal branch to m. quadratus labii superioris. (*c*) In five bundles of infraorbital nerve fibres in the branch to m. quadratus labii superioris. (*d*) Combined findings given in *b* and *c*.

D. *Muscular branches and intramuscular nerves.* Muscular branches from the facial nerve were examined in animals 2–7 inclusive and in 10 and 11. In each animal intact myelinated fibres from the trigeminal were found to enter the muscle, although each individual branch and bundle of supply did not necessarily contain normal fibres. The upper buccal nerve supplying the quadratus labii superioris muscle of animal 11 is shown in Pl. 2, fig. 7 and Pl. 3, fig. 8. Intact intermingled fibres in that nerve were counted and measured (Table 4 and Text-fig. 4*b*). It will be seen that although these were unimodal in their distribution, nineteen fibres measured over 12–22 μ in diameter. Some intact bundles of infraorbital origin within the muscular nerve were also measured (Table 4 and Text-fig. 4*c*). Fifty-three out of this sample of 411 infraorbital fibres measured over 12 μ in diameter and the largest were 16 μ in diameter (Text-fig. 4*d*). When the findings in this sample were combined with those of the upper buccal nerve, 72 out of 606 fibres entering the m. quadratus labii superioris were found to be over 12 μ in diameter.

When the intact fibres were followed distally into the intramuscular plexuses, some could be traced to overlying skin, mucous membrane, vibrissae and blood vessels (Pl. 3, fig. 10) (Bowden & Mahran, 1956).

DISCUSSION

Examination of Text-fig. 1, which represents findings in thirty-two nerves, shows that no communications were found between branches of the ophthalmic division of the trigeminal and the facial; the communications between the infraorbital and facial nerves are limited to the upper buccal branch of the facial. However, it is clear that fibres of the mandibular division of the fifth enter both the upper and lower divisions of the seventh and that the latter receives additional contributions from the third division through the buccal, the nerve to mylohyoid and the mental nerve.

The histological evidence presented here shows that in the rabbit the communications between the two nerves consist of fibres from the trigeminal and that they do not contain any fibres from the facial. As no sympathectomized animals have been studied, the presence of sympathetic fibres cannot be excluded.

The statements of Huber (1930) and O'Connell (1936) that trigeminal nerve fibres travelled with the branches of the seventh to supply blood vessels and overlying skin and its appendages are confirmed; but whilst it is true that some fibres of trigeminal origin are distributed to the periphery without intermingling in any way with those of the seventh, there is clear evidence that some fibres from both nerves take part in forming intraneural and intramuscular plexuses in the rabbit. The finding of these intraneural and intramuscular plexuses is of interest. O'Connell (1936) suggested that in mixed nerves and so-called motor nerves, the intraneural plexuses served to group afferent and efferent fibres together. In discussing the significance of intramuscular plexuses, Feindel, Hinshaw & Weddell (1952) pointed out that plexus formation might be the anatomical basis for scattered, rather than compactly grouped, muscle fibres in a single motor unit, and whilst they did not comment on the possibility of grouping motor and sensory fibres together, it is clear that it is not excluded.

The fact that classical spindles and the spiral type of sensory ending have been found in the facial muscles of man (Kadanoff, 1956) and in m. quadratus labii superioris of the rabbit (Bowden & Mahran, 1956) has put the question of an afferent supply to these muscles beyond dispute for these two species. It remains to discover the source of these afferent nerve fibres. As discussed by Mahran (1955), histological, experimental, electromyographical and clinical evidence has been marshalled in support of each of two conflicting views on their origin either from the trigeminal or the facial nerves. The afferent fibres from skeletal muscles range from 1 to 20 μ in diameter, with the spindle afferents in the 12–20 μ group. The motor supply of the intrafusal fibres is certainly derived from the gamma efferents (3–8 μ diameter) and possibly from the larger motor fibres of 8–12 μ diameter (Barker, 1948; Cooper & Daniel, 1956).

The previous investigation (Bowden & Mahran, 1956) showed that spiral endings were formed from fibres of varying diameter; the fibres forming the equatorial spiral endings in three out of the four spindles reported were measured subsequently, and found to be approximately 20, 20 and 15 μ in diameter. However, this observation must be interpreted with caution for the gold chloride technique which was used stained the axons and gave only a faint indication of the outer limit of the sheath and the fibres were measured in longitudinal and not in transverse section. A motor fibre which ran with one of these afferent fibres and then went on to supply adjacent extrafusal fibres in the same section measured 10 μ. Whilst these measurements cannot be regarded as free from uncertainty, they indicate that fibres supplying the nuclear bag region are in the larger size range. There has been a tacit assumption that if spindles existed in the facial muscles, their pattern of innervation would resemble that found in limb and trunk musculature, and since measurements of facial nerve fibres failed to reveal the presence of any in the larger size range, it has been suggested that there were either very few or no spindles in mimetic musculature. The size-frequency distribution of fibres in the facial nerve trunk and posterior auricular nerve reported here is in general agreement with the findings of Wakeley & Edgeworth (1933) who measured the myelinated fibres in the rhesus monkey just distal to the stylomastoid foramen, and also with those of Haggqvist (1938), Bruesch (1944) and van Buskirk (1945) who measured the diameter of fibres in the facial nerve of the cat and found that they ranged from 1 to 11 μ in diameter. It is not clear whether the last three workers made their measurements proximal or distal to the communications with the trigeminal nerve which were not mentioned but are known to exist in the cat (Mahran, 1955).

Fibres derived from the mandibular division of the trigeminal nerve entered the branches supplying m. quadratus labii superioris and included some of 12–22 μ diameter. In addition, fibres of 12–16 μ in diameter entered the nerves and muscles from the infraorbital branches of the trigeminal. Foley & Dubois (1943) working on cat and dog, Bruesch (1944) working on the facial nerve and its muscular branches in the cat and Fernand & Young (1951) working on a nerve to m. depressor labii inferioris in the rabbit, made no mention of communications with the trigeminal, and found no fibres larger than 12 μ in diameter. At first sight it is difficult to reconcile the findings of these earlier workers with the present observations. A species difference might be invoked as an explanation of the disagreement in the cases of Foley & Dubois and Bruesch, but Fernand & Young were working on the rabbit. The latter measured the fibres in one branch to the m. depressor labii inferioris and in this investigation at least three nerves were found to supply this muscle. It is possible that the large fibres might have entered through another branch, for one of the branches to buccinator studied here after cutting the seventh nerve, was found to be devoid of intact trigeminal fibres, whilst the remaining branches of supply contained them.

The peripheral and central connexions of the large fibres are not yet known. It might be suggested that they supply vibrissae. However, this does not seem likely, since the five large vibrissae studied here had no nerve fibres above 10 μ in diameter, and the diameter of these fibres was not significantly different 1·0 cm. proximal to the theca. Large fibres supply Pacinian corpuscles, but so far none has been observed in the facial muscles, and they are uncommon in limb and trunk musculature. There is some evidence that these large fibres form the annulo-spiral endings in the m. quadratus labii superioris; a total of nineteen fibres of 12–22 μ entered this muscle from the mandibular division in animal no. 11 and, in addition, there were at least fifty-three from the infraorbital nerve which measured 12–16 μ. The number of spindles in this muscle is at present unknown. The central connexions presumably would enter the brain stem with the fifth nerve, and if the pattern of proprioceptive innervation of the facial muscles followed that of the muscles of mastication, the cells of origin of these sensory fibres would be in the mesencephalic nucleus of the trigeminal nerve.

The efferent supply of the intrafusal muscle fibres, on purely morphological grounds of fibre size, might be derived from the seventh or fifth nerves. In this context it is interesting to note that Lewy, Groff & Grant (1938) reported chromatolytic changes in the three parts of the mesencephalic nucleus of the fifth nerve after cutting the seventh nerve in the Fallopian canal in the cat. Although they considered this to be evidence of damage to autonomic fibres, another interpretation might be given, namely, that the motor fibres arose in this nucleus and passed out with the trunk of the facial nerve to intrafusal fibres in facial muscles. Should these hypotheses be correct, both the trigeminal and facial nerves would then be involved in the proprioceptive innervation of the facial muscles, and this would resolve the conflict between the two views which have been discussed. Thelander (1924), Kimmel (1941) and Pearson (1947, 1948 and 1949*a*, *b*) have demonstrated synaptic relationships between the mesencephalic and facial nuclei in the cat, rabbit and man respectively. However, until the effect of various operative procedures upon the spindles themselves is known, the full functional significance of the peripheral and central connexions between these two cranial nerves cannot be assessed.

SUMMARY AND CONCLUSIONS

1. The number and diameter of myelinated nerve fibres in the facial nerve trunk just distal to the stylomastoid foramen were measured in two animals.

The size-frequency distribution was unimodal and ranged from 1 to 12 μ diameter. Similar findings were made in the post-auricular nerve of one animal.

2. The nerve fibres supplying five mystacial vibrissae were counted and measured. These also were unimodal in distribution and no fibres larger than 10 μ in diameter were found.

3. In nine animals the right facial nerve was cut below the stylomastoid foramen and the animals were left to survive for periods ranging from 7 to 53 days after

operation. In all animals the facial paralysis was complete and there was no evidence of re-innervation of the distal stump.

4. Fibres of the trigeminal nerve were followed through the combined nerve trunk and branches.

5. The communications between the fifth and seventh nerves were derived wholly from the former and contained myelinated nerve fibres ranging from 1 to 22 μ diameter.

6. Some of the trigeminal fibres left the combined nerve trunk without change, others intermingled with the denervated bundles of the seventh nerve and formed intraneural and intramuscular plexuses.

7. Some intact trigeminal fibres were traced to skin, mucous membrane, mystacial vibrissae and the adventitia of intramuscular blood vessels.

8. The significance of finding large diameter fibres (12–22 μ) in the branches supplying muscles is discussed.

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EXPLANATION OF PLATES

(Figs. 1 and 6–8 are mosaics.)

PLATE 1

Fig. 1. Transverse section of facial nerve trunk (animal 2) below the stylomastoid foramen. 5 μ thick, stained with osmic acid (× 100).

Fig. 2. Transverse section through the theca (*a*), sinus (*b*) and vibrissa (*c*) of animal 2, showing bundles of myelinated nerve fibres and a few isolated myelinated nerve fibres (e.g. at ↑) within the sinus. (These constitute vibrissal nerve no. 5.) 5 μ thick, stained with osmic acid (× 100).

Fig. 3. Animal 4 showing signs of complete right-sided facial palsy.

Fig. 4. Transverse section of a degenerating bundle of VIIth nerve fibres (*a*) approaching one mostly containing intact auriculotemporal nerve fibres (*b*), from animal 10, 14 days post-operative. 15 μ thick, stained with osmic acid (× 165).

Fig. 5. Bundles seen in fig. 4 now in same sheath (× 165).

PLATE 2

Fig. 6. Transverse section of right upper buccal nerve of animal 11 (28 days post-operative) distal to the communication with the auriculotemporal nerve. Note: the intermingling of intact Vth nerve fibres within large and small bundles of degenerated VIIth nerve; the convergent bundles at *a*; two mixed fasciculi within a single sheath at *b*; large diameter fibres (22 and 20 μ) at *c*. 5 μ, stained with osmic acid (× 100).

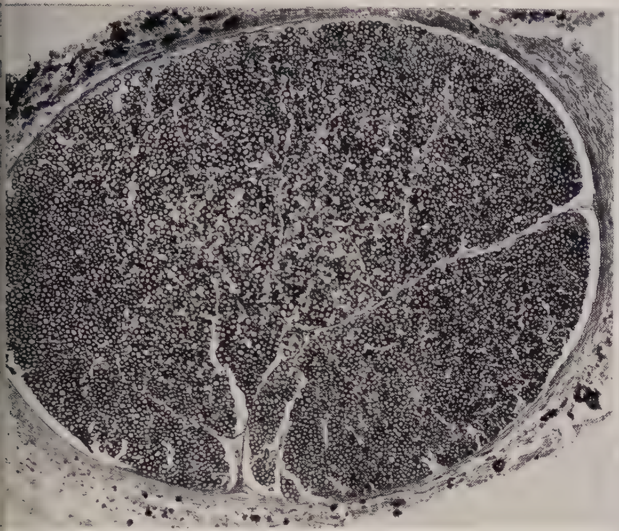
Fig. 7. Transverse section of right upper buccal nerve (animal 11) supplying m. quadratus labii superioris (*a*). Note intact bundles from the infraorbital nerve (*b*); mixed bundles with degenerated VIIth nerve and intact trigeminal fibres in the remainder of the section. 5 μ, stained with osmic acid (× 100).

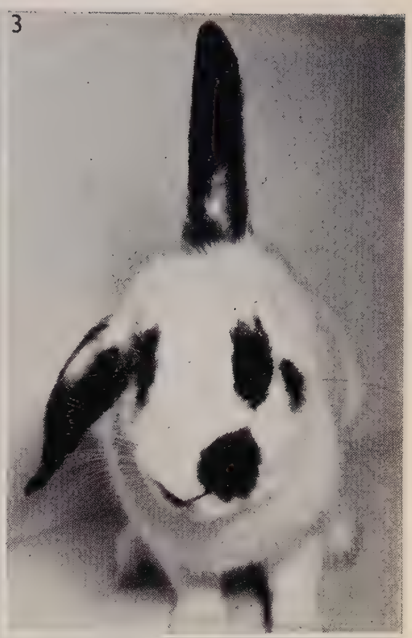
PLATE 3

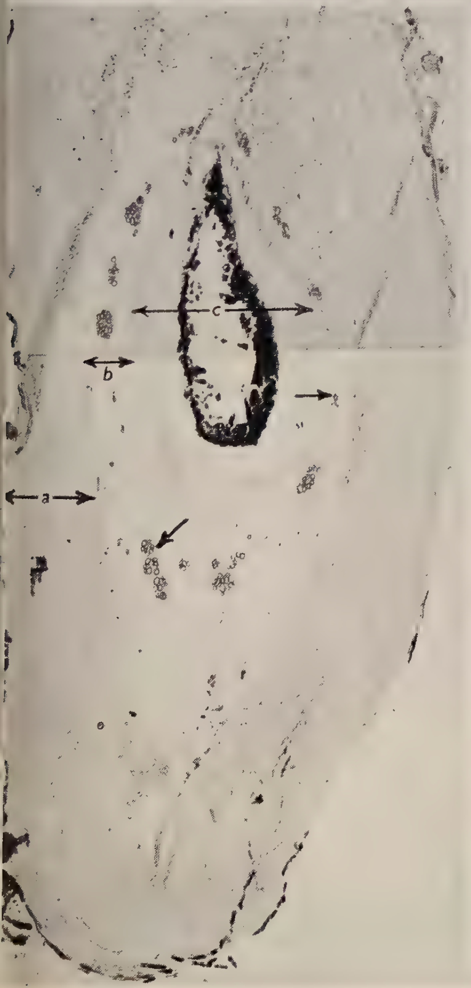
Fig. 8. More distal transverse section of nerve shown in fig. 7. The intact bundles (*a*) from the infraorbital nerve entered m. quadratus labii superioris and the fibres were counted and measured. 5 μ, stained with osmic acid (× 100).

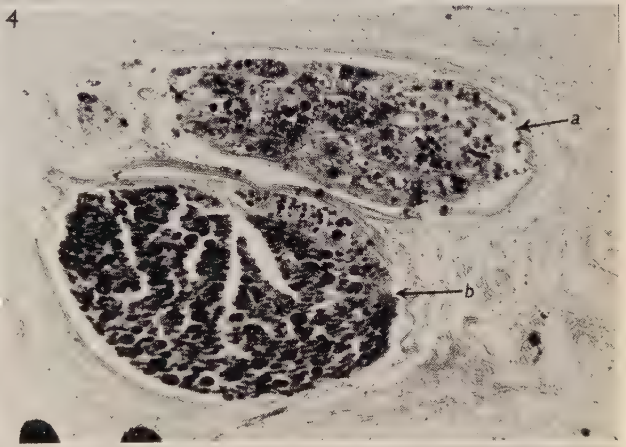
Fig. 9. Section taken between those shown in figs. 7 and 8 from the nerve to quadratus labii superioris. Note intact bundle (*a*) and small bundle (*b*) containing intact and degenerated fibres. 5 μ, stained with osmic acid (× 750).

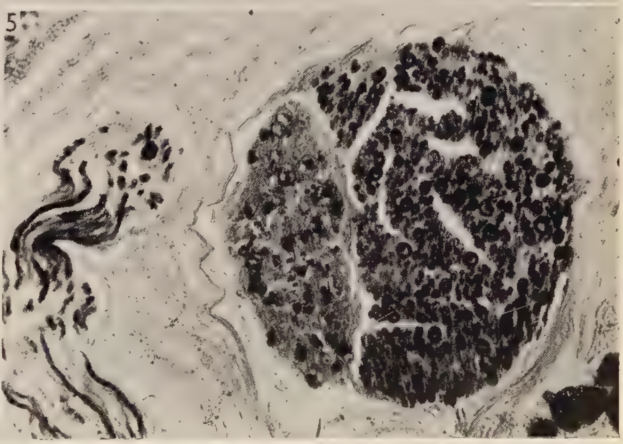
Fig. 10. Healthy branch of infraorbital nerve (*a*) sending intact fibre, (*b*) into a degenerating bundle of VIIth nerve fibres (*c*) in the intramuscular plexus of m. quadratus labii superioris, animal 10, 14 days post-operative. Gold chloride stain (× 57).











BOWDEN AND MAHRAN—STUDIES OF THE EXTRAPETROUS PORTION OF THE FACIAL NERVE

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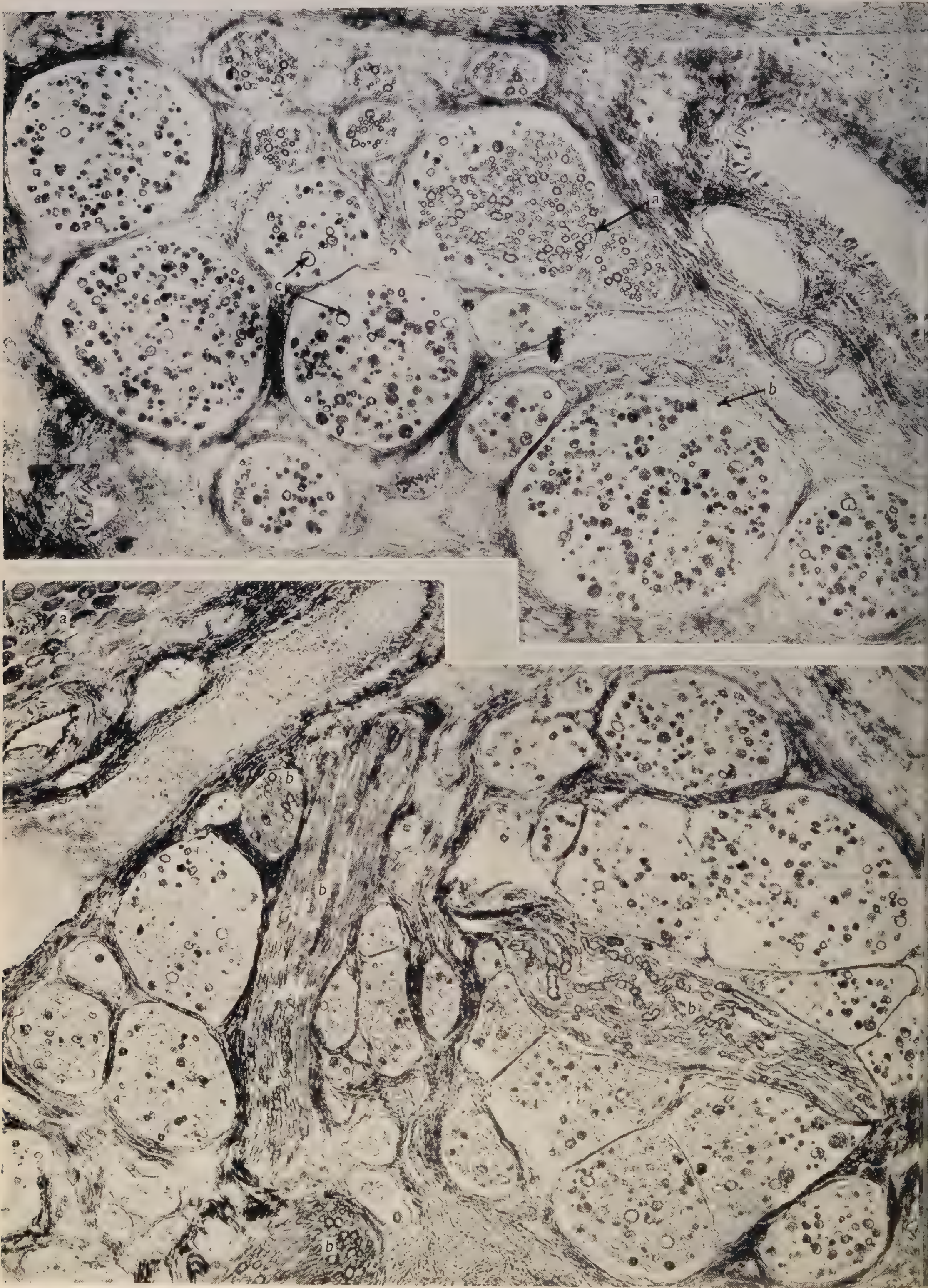
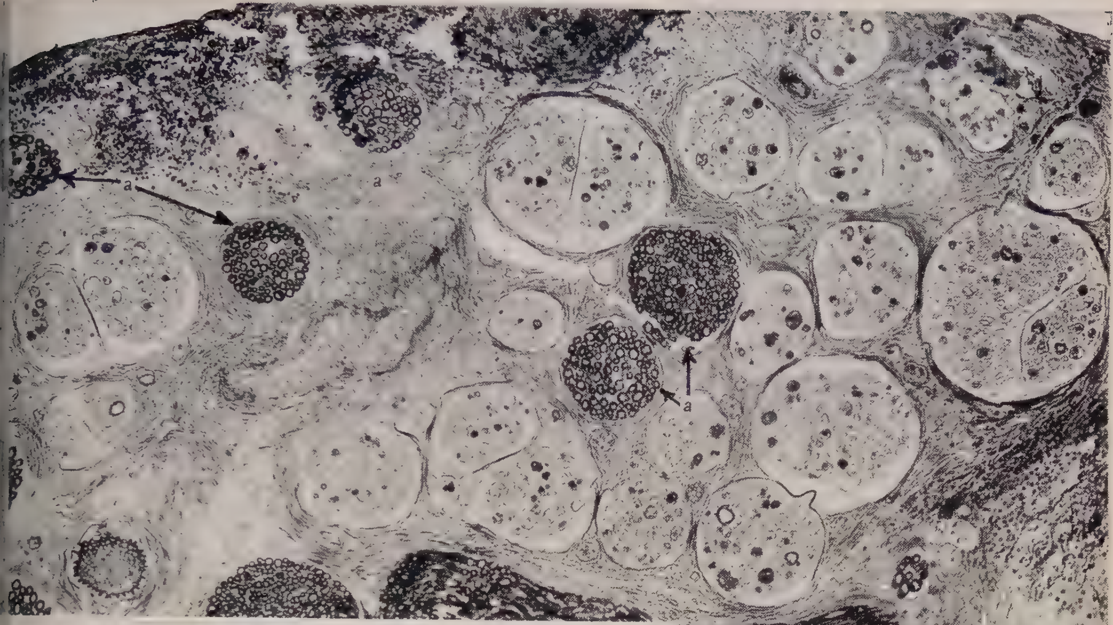
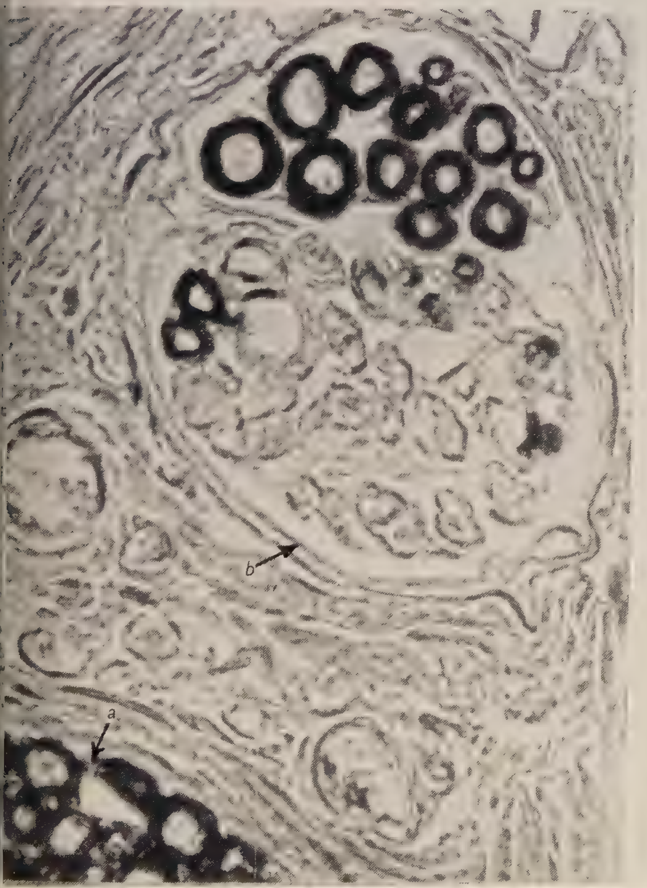
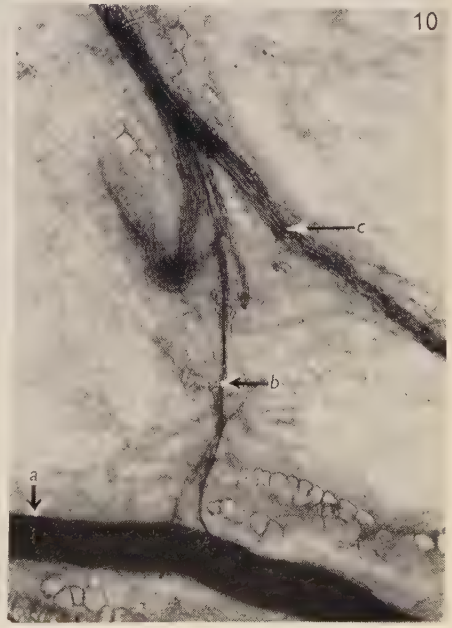


BOWDEN AND MAHRAN—STUDIES OF THE EXTRAPETROUS PORTION OF THE FACIAL NERVE







BOWDEN AND MAHRAN—STUDIES OF THE EXTRAPETROUS PORTION OF THE FACIAL NERVE

AN ELECTRON MICROSCOPE STUDY OF THE CORNEA IN MICE, WITH SPECIAL REFERENCE TO THE INNERVATION

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INTRODUCTION

The cornea is a tissue which has been investigated very fully by the methods of light microscopy. The innervation of the cornea is discussed at length by Zander & Weddell (1951) who review the earlier literature. Electron microscope studies dealing with the structure of the cornea, but not with its innervation, include Rouiller, Danon & Ryter (1954), Jakus (1954, 1959) and Sheldon (1956). The present investigation was undertaken to clarify some problems of the relationships of nerve fibres to cells of the epithelium and the stroma, and to obtain pictures of the fine structure of sensory nerves which have free endings.

MATERIAL AND METHODS

Mice were chosen because their eyes are small enough to allow the cornea to be fixed whole, and to be embedded when cut into only a few pieces, which can be orientated; sections can then be made in particular known planes. Also, the plexiform arrangement of the nerve bundles is simpler than in larger animals, though essentially similar. The mice used were young adults of pure lines A and CBA and of various hybrid stocks. Pathological conditions of the cornea are common in laboratory mice; any eyes that appeared to be ulcerated or otherwise abnormal were discarded.

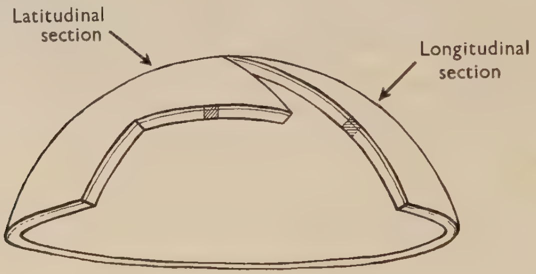
Fixation, for electron microscopy, was with 2 % osmium tetroxide mixed with an equal volume of 0·85 % saline buffered at pH 7·4, kept on ice throughout. The mice were killed with injections of nembutal; as soon as an animal was dead, drops of osmium tetroxide were placed on the eyes. The eyes were protruded by pressure on the neck, and the corneae cut off with a razor blade, dropped momentarily into saline so that the iris could be removed, then transferred to a bath of the cold fixative for 3 hr. After washing in distilled water, each cornea was cut into quadrants with a sharp blade. As the unstained tissue proved to show very little contrast, the pieces were stained in a 1 % aqueous solution of phosphotungstic acid for 1, 2 or 3 hr. Dehydration was with ethyl alcohol in the usual way. The specimens were embedded either in a mixture of 15 % methyl- and 85 % butyl-methacrylate, or in Araldite epoxy-resin (see Glauert & Glauert, 1958).

Attempts made to fix the cornea in potassium permanganate were unsuccessful, because, in spite of various modifications of technique, the epithelium always disintegrated.

Ultra-thin sections were cut with a Porter–Blum microtome and a glass knife;

they were usually treated with chloroform vapour before being transferred to the grid, to counteract the compression effect of cutting (see Sotelo, 1957). The microscope was a Siemen's Elmiskop I. There were three principal planes of section. The cornea of a mouse is roughly hemispherical: sections were cut either vertically to the surface and in the planes of latitude or of longitude, or tangentially to the surface. In Text-fig. 1, the shaded areas represent the maximum size of a locality which could be explored in any one series of sections; usually the block face was of about one-quarter this area.

In the Plates, all latitudinal and longitudinal sections are orientated so that the epithelium, or the outer surface of the cornea, would be towards the top of the page; this direction is referred to as 'upper'. The tangential plane of section, not illustrated in the figure, passed through the epithelium obliquely instead of vertically; the sections were only approximately parallel to the outer surface, and have not been orientated on the page.



Text-fig. 1. Diagram of the cornea to show two of the planes of section. Magnification approximately 20×.

It was obvious that sections of specimens embedded in methacrylate had suffered considerable shrinkage, either in the block or while in the electron beam; estimates of the changes in volume taking place during fixation, embedding, etc., are made by Bahr, Bloom & Friberg (1957). Sections of araldite blocks did not shrink as noticeably in the beam, and Glauert & Glauert (1958) claim that there are no shrinkage effects in the block. Phase contrast photomicrographs of araldite material, taken with a $\frac{1}{12}$ in. oil-immersion lens, were compared with similar photomicrographs of optical sections of whole mounts of fresh corneae. There was no obvious difference in the diameters of the epithelial nuclei which were measured in the two sets of photomicrographs; this is a crude method of estimating possible changes in volume during embedding, but at least it indicates that shrinkage could not have been pronounced.

Some distortion from compression during cutting is unavoidable, even if the sections are treated with chloroform vapour. So as to be able to compare sections from different blocks, all latitudinal and longitudinal sections were cut with the block held so that the epithelium in the specimen was horizontal. If the epithelium was vertical in the block, and so at the side of the ribbon of sections, it was more difficult to follow a particular structure through a long series of sections. Therefore, the horizontal orientation was used throughout, in spite of the disadvantage, mentioned later, that it may have made the figures for diameters of nerve fibres somewhat too low.

Any structure under investigation was followed through as many sections as possible; the numbers of serial sections available varied widely according to circumstances.

Most measurements given are taken from prints of electronmicrographs of araldite sections, at a magnification of either 15,000 or 7,000 times, enlarged photographically 3·5 times. Some measurements were made at greater magnifications. Measurements from methacrylate material were not used. All the figures in the Plates are from araldite sections, except Pl. 2, fig. 19, which is in methacrylate.

In addition to the material examined with the electron microscope, numerous microscopical preparations of mouse cornea were made by supravital staining in 0·01 % methylene blue; toluidine blue and brilliant cresyl blue were used supravitally to a limited extent. Also, the corneae of a few CBA mice were tested for the dihydroxyphenylalanine (DOPA) reaction, and fresh corneae in saline were examined by phase contrast microscopy.

GENERAL DESCRIPTION OF MOUSE CORNEA

The total thickness of the cornea is about 100 μ, of which some 30 μ is epithelium and some 65 μ, stroma. There is a Descemet's membrane and an endothelium, but no Bowman's membrane. In the epithelium there is a basal layer, one or two layers of cells in the middle, and four or five or more layers of flattened cells on the outside. The innervation of the cornea in mice has the form described for other mammals; any minor points of difference will be mentioned where appropriate. Zander & Weddell (1951) should be consulted for photomicrographs of corneal nerves as they appear in methylene blue and in silver preparations.

In electronmicrographs of the epithelium, adjacent cells are seen to be linked by interdigitating processes, as described by Jakus (1954). Both Jakus, in that paper, and Sheldon (1956) show micrographs of sections where there are large extracellular spaces between the epithelial cells. Such spaces were not seen in the present material, except in the most superficial flattened layers, and elsewhere when sections had been taken from near the edge of a piece of cornea; here there was reason to suspect that the specimen might have suffered mechanical damage when the cornea was cut up. Normally the cell membranes of adjacent cells were separated by the usual gap of 200–300 Å.

On the cell membranes of the epithelial cells occur numerous pairs of attachment plaques or desmosomes resembling those described by Odland (1958) and by Hibbs & Clark (1959) in human epidermis. Jakus (1959) has also found these plaques in the corneal epithelium of various vertebrates. In mice, the plaques were most numerous on the walls of the middle layer cells (a fact useful for the identification of these cells in tangential sections) and on the lower wall of the basal layer cells. On the lateral walls of the basal layer cells attachment plaques are scarce, though they do occur. They were also seen in the lower flattened layers, but were there less conspicuous than in the deeper levels of the epithelium. Plaques from the middle layers of the epithelium are illustrated in Pl. 1, figs. 1 and 2, and from the base of the epithelium in Pl. 1, fig. 3. They have a close resemblance to those of human epidermis, but are smaller. Each plaque consists of a disc of thickened cell membrane of diameter about 0·1 μ in the middle layers and about 0·15 μ at the base of the epithelium. Within the cytoplasm, adjacent to the disc, is a more or less conical dense region, the tonofilament area. In Pl. 1, fig. 2 the depth of the darkest part, which is the thickened cell membrane, is about 150 Å, which is twice that of the normal cell membrane. (It should be noted that in araldite sections of osmic-fixed material the double structure of the cell membrane is not usually apparent, although it does show in osmic-fixed methacrylate embedded specimens.) In Pl. 1, fig. 2, the thickened part of the cell membrane does not appear homogeneous, but no details of structure can be made out. Immediately outside the thickened cell membrane is a light zone, corresponding to the proximal light layer of Odland, which in Pl. 1, fig. 2 is something over 20 Å. in depth. Next to this is a denser layer of depth about 60 Å., corresponding to the intermediate dense layer of Odland. The intermediate dense layers of opposite sides here approach one another closely, and no structure corresponding to the intercellular contact layer of Odland could be made out. Hibbs & Clark (1959) say that the central lamellae of their extracellular desmosomal material did not always appear. At the base of the epithelium a reliable estimate of the depth of the thickened cell membrane could not be made, because it was difficult to see where the membrane ended and the tonofilaments began. All the other dimensions were somewhat larger than in the middle layers, the light zone being 40–50 Å. deep, the intermediate dense layer from 60 to 100 Å. deep. This extracellular dark line here lies within the pale zone separating the basal cell membrane from the 'basement membrane' of the epithelium. The thickness of the basement membrane is usually about 300 Å., the depth of the pale zone the same or somewhat greater. Occasionally, round dark bodies were seen intervening between the basal cell membrane and the basement membrane; these were apparently the ends of processes from adjacent basal cells.

The cytoplasm of the basal and middle layer epithelial cells appears dark and granular in PTA-stained sections. It is fibrillar, especially so where there are cell processes, and occasionally shows vacuolar profiles of up to $\frac{1}{2}$ μ in diameter. There are also mitochondria, mostly near the nucleus; these are often elongated and rather irregular. In the cells of the middle and basal layers it was not easy to make out the membranes of the mitochondria, especially the outer membranes. Pl. 1, fig. 4 shows the typical appearance of a mitochondrion in the middle layer; the broad clear area round the central part was always present in mitochondria of the middle and basal layers of the epithelium. In the flattened layers, where the cytoplasm appears less dense and more fibrillar, there is the appearance of an outer membrane round the mitochondrion, separated from the inner part by an irregular gap; this is illustrated in Pl. 1, fig. 5. It might be argued that the peculiar appearance of the epithelial mitochondria is an artefact due to bad penetration of the fixative, but the mitochondria in the nerve fibres and in other cell types of the epithelium (see below) are orthodox in structure.

The stroma or *substantia propria* consists of collagen and of stromal cells, or corneal corpuscles, which are large flattened cells with branches extending horizontally, that is, parallel to the surface of the cornea. The collagen fibrils are arranged in lamellae with the fibres of alternate layers running at right angles to one another. 'Corneal spaces' around the stromal cells do not appear in electron micrographs. Near their nuclei the stromal cells show a rich endoplasmic reticulum appearing either as numerous annular membranes or as long parallel membranes; there may also be cisternae up to $\frac{1}{2}\,\mu$ in diameter. A portion of a stromal cell, near its nucleus, is shown in Pl. 1. fig. 6. The mitochondria have orthodox parallel cristae, but there are sometimes 'target organs' with concentric or spiral lamellae (see Robertson, 1959).

The stromal cells are usually said to form a syncytium; certainly the branches of adjacent cells are in conjunction. This accounts for a common appearance of the flattened parts of the cells when seen in vertical sections: often they appear to be split horizontally. In such a case processes from two cells are generally present, although sometimes the infolding of the surface of a single cell may produce a similar appearance. The gap between the membranes of adjacent stromal cells is usually from 200 to 500 Å. A section across the flattened extensions of two stromal cells is shown in Pl. 1, fig. 7. Besides the flat sheet-like extensions, the cells give off small irregular branches about 0·1 μ in diameter, which extend for considerable distances into the collagen lamellae above and below the cell, and may be encountered almost anywhere among the collagen, although not in great numbers; one is illustrated in Pl. 1, fig. 8.

Near the periphery of the cornea there are larger spaces between some of the stromal cells, as in Pl. 1, fig. 9. Very near the limbus may be found spaces, one of which is as shown in Pl. 1, fig. 10; this space has a total breadth of more than 10 μ and a maximum depth of 2 μ. Two cells are involved in the walls, one above, and one below, which shows its nucleus; the cells interdigitate at the sides, as can be seen on the left. The cells forming the walls of spaces such as this have highly vacuolar cytoplasm; some of the vacuoles appear to open into the lumen of the space. Fine projections, about 500 Å. across, extend at intervals from the walls into the space. Spaces between stromal cells, intermediate in size between those of Pl. 1, figs. 9 and 10, have also been seen. The possibility that the largest spaces are blood capillaries cannot be excluded, but no blood corpuscles were seen in them, although several were followed in serial sections. The smaller, and more irregular, spaces cannot be blood capillaries, and it is unlikely that they are artefacts, because they were not seen in the central parts of the cornea. It is tempting to suppose that both systems of channels are interconnected and are concerned with the movement of fluids into, or out of, the cornea; in the central part of the cornea, fluid movement must be affected by the stromal cells.

THE NERVES IN THE STROMA

Nerve bundles enter the cornea radially at the limbus; each contains a few myelinated fibres and a number of finer unmyelinated fibres. Just within the limbus the bundles divide and form a plexus which extends throughout the stroma. In mice the plexus is disposed in three main layers; the deepest of these is sparse and consists of little more than a bundle running round the circumference of the cornea not far from the limbus. Branches of this deep peripheral bundle rise to the middle layers of the stroma, and form a plexus extending over the whole breadth of the cornea. From this mid-stromal plexus branches rise again to the upper layers of the stroma to form the subepithelial plexus; this occupies the upper 10 μ of the stroma but is not all strictly at one level.

It is usually stated that medullated nerve fibres lose their myelin soon after entering the cornea, but in mice myelinated fibres may be found even towards the centre of the cornea in the mid-stromal plexus, though not above this level. Such fibres may be seen in methylene blue preparations, but the presence of myelin was also confirmed in electron micrographs. A similar observation was made by Rodger (1951) in cats, with a polarizing microscope. In mice, the large deep peripheral bundles usually contain two or three myelinated fibres, while the large bundles of the mid-stromal plexus may contain one or two.

For electron microscopy, the deep peripheral bundles may be found if longitudinal sections are cut near the limbus. Like most of the other stromal bundles, they are flattened and strap-like, and are, in transverse section, too broad to be included in a single block face. Bundles in this position have a sheath which is lacking in other stromal nerves. Each myelinated fibre or group of unmyelinated fibres is wrapped in a Schwann cell in the usual way; outside the Schwann cell and separated from it by some collagen fibrils is a sheath of a single layer of cells which in electron micrographs appear similar to the ordinary stromal cells. The cells of the outer sheath intrude between adjacent Schwann cells as shown in Pl. 1, fig. 11; in this micrograph there is a part of a myelinated fibre on the left, and on the right a Schwann cell, with nucleus, enclosing a group of unmyelinated fibres.

The most superficial stromal nerve bundles near the limbus may also be atypical of the cornea as a whole. Possibly they should not be called corneal bundles, as the epithelium here is already changing its character; Zander & Weddell (1951) state, however, that the small bundles entering the cornea from the pericorneal plexus do penetrate the stroma or the corneal epithelium and mingle with the nerves which enter the cornea at a deeper level. In a longitudinal section of the corneal limbus, a few stromal bundles may be found indenting the base of the epithelium, a position which stromal bundles never occupy in more central parts of the cornea. Pl. 1, fig. 12, shows such an indentation of the epithelium, containing collagen fibres and two bundles of nerves, each with Schwann cell cytoplasm; one has two and the other three nerve fibres. As is usual, a basement membrane follows the contour of the epithelial cells and also surrounds the nerve bundles. What is peculiar is that there appears to be a continuous sheet of basement membrane across the floor of the channel in the epithelium, in the plane of the general base of the epithelium; this curious phenomenon was seen under all those stromal nerves which indented the epithelium. In Pl. 1, fig. 12, there is a nerve fibre on the extreme left, between the epithelial basement membrane and the basal cell membrane, which is presumably an epithelial fibre derived from the adjacent stromal bundle; the serial sections of the area which were available did not show where this happened. (The dark line running diagonally across the right-hand nerve bundle is a shadow, an artefact of this section.) The upper layers of the stroma at the periphery also contain numerous other bundles of unmyelinated fibres which are not different from those in the central parts of the cornea.

The mid-stromal plexus contains both large strap-like nerve bundles, often with one or two myelinated fibres, and smaller bundles which may or may not be flattened and contain small unmyelinated fibres only. The smallest bundles were not at all obvious on the screen of the electron microscope, and might easily be missed when scanning a section. All nerves at this level have the Schwann sheath only, with no external sheath of other cells. In one series of sections there were a few single unmyelinated fibres wrapped in Schwann cell cytoplasm but separated from the other fibres of the bundles. An example is shown in the record of fibre diameter in Text-fig. 6*b*. There were two nerves containing such isolated fibres; most of the fibres were of about the same diameter as the one in Text-fig. 6*b*, but two were only half the size. It was presumed that the isolated single fibres had been myelinated but had not yet come to share a Schwann cell with other fibres; there was no direct evidence for this, but the condition was not seen except in the mid-stromal plexus, where the medullated fibres do lose their myelin sheaths. Nerves of the mid-stromal plexus are not illustrated as their appearance is orthodox; the smallest bundles usually look like that in Pl. 2, fig. 13, which is from the subepithelial plexus.

In the subepithelial plexus there are no myelinated fibres. In microscopical preparations the branches of previously medullated fibres can be traced into the subepithelial plexus; in electron micrographs there can be no direct evidence as to whether a particular fibre was or was not medullated when it entered the cornea, but it can be assumed that the larger fibres are branches from medullated fibres. Pl. 2, fig. 14 shows part of a large flattened nerve bundle 2 or 3 μ below the base of the epithelium; the total breadth of this bundle was about 10 μ, and it contained thirteen fibres, whose diameters are recorded in Text-fig. 8*b*. Each nerve bundle is wrapped in a Schwann cell, but the Schwann cell cytoplasm does not necessarily penetrate between all the fibres; the five fibres on the right of Pl. 2, fig. 14 are not separated by Schwann cytoplasm, nor are the two middle fibres in Pl. 2, fig. 13.

Not all the bundles of the subepithelial plexus are strap-like: Pl. 2, fig. 15 shows a nerve from a deeper level than that of Pl. 2, figs. 13 or 14, below the third layer of stromal cells from the epithelium. This nerve was followed in thirty serial, but not consecutive, sections; the diameters of its fibres are recorded in Text-fig. 7*b*. Here also a single Schwann cell envelops the nerve fibres, but not all are separated from one another, nor are all covered on the outside by a layer of Schwann cytoplasm. Examination of serial sections shows, however, that the Schwann cell, at other places, pushes between the nerve fibres which are in apposition here, and also covers the fibres which are naked on the outside of the bundle in Pl. 2, fig. 15.

Nerve fibres of large and small diameter, and from any bundle, may contribute to the innervation of the epithelium, but certain fibres terminate in the stroma (see Zander & Weddell, 1951; Ábrahám, 1955; and many other authors.) According to Zander & Weddell, fibres leave the bundles and travel long distances in the stroma, apparently naked. Ábrahám states that in mammals many nerve fibres are closely associated with stromal cells and appear to terminate within them. There is variation in different species: in mice, fibres apparently ending in the stroma can be found in methylene blue preparations, mostly just beneath the epithelium. A search made with the electron microscope to find such fibres and to determine whether or not they are devoid of Schwann cytoplasm encountered a difficulty, in that in transverse sections of nerves it was not always possible to distinguish between axons and Schwann cytoplasm in the micrographs, even when serial sections were available. In such a nerve as that of Pl. 2, fig. 15, some structures which appeared to be axons in certain sections were found to be lobules of Schwann cell when the complete series was examined. It is even less easy to differentiate in the small bundles containing only three or four fibres. Pl. 2, fig. 16 shows an example: serial sections show that some Schwann cytoplasm is present in this nerve bundle, but it is impossible to tell in the section illustrated which parts are nerve and which are sheath. When a nerve fibre is cut lengthwise, it can be distinguished from a Schwann cell by the neurofilaments, but then a Schwann cell may be present without being included in the sections available. In the upper levels of the stroma some structures were followed which appeared to be fibres which left nerve bundles and ran naked among the collagen fibrils, but it was not possible to be sure that they were nerves. It must be understood that a long series of sections is not easy to obtain, particularly a complete one, nor can one be quite certain of the order of the sections except over a short distance. The plexiform arrangement of the fibres increases the difficulty of serial identification. Small bundles of a few nerve fibres are common in the upper levels of the stroma, and although some of these certainly enter the epithelium, others may represent the fibres which appear solitary in methylene blue preparations.

The question of the naked stromal endings must then be left open, except that it can be stated that in the upper layers of the stroma nerve fibres, even those which will enter the epithelium, are not necessarily enclosed in the Schwann cell for the whole of their course; they may lie on the surface of the Schwann cell, like the lower fibre of Pl. 4, fig. 28, or at the edges of the bundle as do the two lateral fibres in Pl. 3, fig. 24. In such cases a 'basement membrane' may intervene between the nerve and the surrounding collagen, just as it usually does between the Schwann cell membrane and the collagen. The dark shadow surrounding the nerve bundles appears very similar in electronmicrographs to that underlying the epithelium, so that it seems legitimate to call it a basement membrane until it is shown to have a different nature. Close examination of Pl. 2, figs. 15, 16 and Pl. 3, fig. 24 will show that the basement membrane is not invariably present over the whole surface of a nerve bundle. A basement membrane was not seen outside the stromal cells, as can be verified in Pl. 1, figs. 6–9; as the stromal cells are responsible, among other things, for forming and maintaining the collagen, one would not expect them to be separated from it.

Although a search for them was made, no nerve fibre was seen running on or in a stromal cell in the manner described by Ábrahám. This does not mean that such fibres do not exist, only that they must be scarce in mice; they were not seen in the methylene blue preparations either. Occasionally in electron micrographs a nerve bundle was seen apparently touching a stromal cell in passing.

IDENTIFICATION OF NERVES IN THE BASAL LAYER OF THE EPITHELIUM

In the epithelium the nerve fibres are devoid of Schwann cells, and in sections there is a possibility of confusing them with various other structures. It happens that in mice most of the nerve fibres in the basal layer of the epithelium run in the longitudinal direction; therefore sections in the latitudinal plane (see Text-fig. 1) will contain more fibres, cut approximately transversely, than will sections cut in the longitudinal plane. By examining stretches of the stromal-epithelial junction in sections cut in the latitudinal and in the longitudinal planes, and in planes intermediate between these, the identity of the nerve fibres at this level was established. The nerve fibres in the basal layer are invaginated into the epithelial cells in deep grooves, as already reported (Whitear, 1957). These fibres may occur singly, or in bundles (leashes) of two or three or up to more than a dozen fibres. The fibres in a leash may be derived from the splitting of a single fibre entering the epithelium, but are not necessarily so. Nerve fibres in the basal layer of the epithelium are shown in Pl. 1, fig. 3; Pl. 2, figs. 17, 18, 20; Pl. 3, figs. 22, 23; Pl. 4, figs. 29, 31. In Pl. 2, fig. 18, serial sections showed that the large fibre on the right is about to divide into two. When micrographs showing basal layer nerve fibres cut lengthwise were needed, they were found mostly in sections cut in the tangential plane, as in Pl. 4, fig. 32.

There is some danger of confusing nerve fibres with the interdigitating processes of the epithelial cells, already mentioned, as they are of a comparable size. However, a cell process which is merely projecting into the cell next to it, not traversing it, is inserted into a pocket rather than running in a groove. A nerve fibre passing right across an epithelial cell can be recognized because the cell membrane embracing it is connected to the side of the cell; this is best seen by reference to the diagram of Text-fig. 2. Where mitochondria are present in a cell process they will serve to differentiate it from a nerve fibre, by the presence of the clear area mentioned above (nerve fibre mitochondria are orthodox in appearance, although elongated). Normally, the appearance of the axoplasm itself is distinctive, since it is paler than the epithelial cell cytoplasm and more fibrillar, but this is not always so, the epithelial cell processes often contain fibrils. Attachment plaques were not seen on the walls of nerve fibres; those near the fibre of Pl. 4, fig. 34 are between two epithelial cells.

THE BASAL LAYER BRANCHED CELLS

There is another possible source of confusion in the identification of epithelial nerve fibres. Peculiar branched cells occur in the corneal epithelium; Text-fig. 3 shows such a cell as it appears after staining with methylene blue, *supra vitam*. Such cells were first described by Egorow (1934, cited by Scharenberg, 1955, and by Pau & Conrads, 1957). Scharenberg calls them polymorph elements, while Pau & Conrads refer to them as Langhans cells, homologizing them with the high-level dendritic cells of mammalian epidermis. Billingham & Medawar (1950) who also illustrate these cells, were of the opinion that they are not Langhans cells; they thought the cells lay immediately below the epithelium, in fact they lie within it. Here, these cells will be referred to as 'basal layer branched cells', so making no assumptions about their homologies. Most of the cells lie within the basal layer of the epithelium, although some of their branches extend through the middle layers, and occasionally a cell body is found above the basal layer.

The basal layer branched cells are easily recognized in electron micrographs; in methacrylate-embedded specimens especially they could not be missed, as the cytoplasm appeared markedly paler than that of the basal epithelial cells. In araldite specimens the pallor was not so pronounced. Being elongated, the nucleus appears smaller, in transverse section, than the nucleus of an epithelial cell. Near the nucleus the cytoplasm contains large mitochondria, which are orthodox in appearance, endoplasmic reticulum and sometimes vacuoles and multivesicular bodies (see Sotelo & Porter, 1959, for references to the literature on these bodies). In the arms of the branched cells the cytoplasm shows fewer contents, as a rule. The basal layer branched cells have been described as syncytial by some authors (Pau & Conrads, 1957); electron micrographs show that a branch of one cell may be intimately associated with the body or branches of another. Pl. 2, fig. 19 shows a transverse section of the body of a basal layer branched cell which is wrapped around a process from another; this has been followed, in several serial sections, from a position on the other side of the cell. The cell membranes are separated by the usual gap, but the two cells are so closely associated that they might appear syncytial by light microscopy. Whether every basal layer branched cell is in contact with others is more doubtful: in some series of sections none was seen, whereas in others there were several included within the block face. There was an impression that the numbers of cells varied in different lines of mice; pure line A white mice could be relied on to show numerous basal layer branched cells, but this may have been coincidental; no quantitative estimate was made.

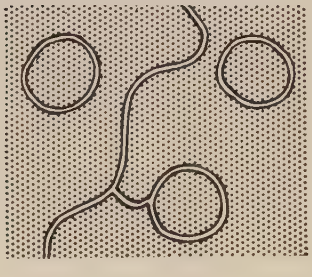


Fig. 2

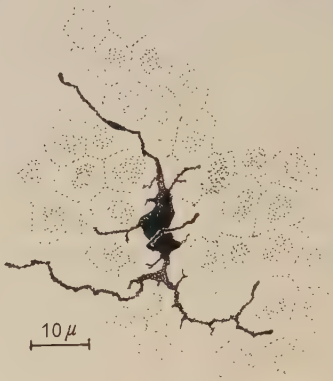


Fig. 3

Text-fig. 2. Diagram to show the appearance of the cell membranes around an epithelial nerve fibre and around two processes from adjacent epithelial cells.

Text-fig. 3. Camera lucida drawing of a basal layer branched cell stained with methylene blue, *supra-vitam*, as seen from above. The outlines of basal layer epithelial cells are indicated.

Certain authors, including Pau & Conrads and Scharenberg, have reported that the branched cells are connected with the epithelial nerve fibres; the former authors regard them as forming a nerve sheath in the manner of a Schwann cell. Certainly it does appear, in preparations seen with a light microscope, that nerve fibres are in contact with the branched cells. The present study has shown that the basal layer branched cells do not in fact have any special relationship to the nerve fibres. Occasionally they may be adjacent, but this is fortuitous; many fibres have been followed where there was no trace of a basal layer branched cell, and often a fibre or fibre bundle has been found near a basal layer branched cell, but separated from it by the cytoplasm of a basal layer epithelial cell. Such a situation is shown in Pl. 2, fig. 20, where a pair of nerve fibres, cut obliquely, lies near, but not in contact with, the body of a branched cell. It was quite the exception to find fibres next to the branched cells, and they were never wrapped up in them. As the nerve fibres and the basal layer branched cells lie at the same level in the epithelium, they must sometimes be near each other.

The basal layer branched cells do not, then, correspond to Schwann cells. What their homologies are, remains obscure. They are most easily demonstrated by staining *supra vitam* in a dilute solution of methylene blue in isotonic saline to which has been added a little sodium bicarbonate; basal layer branched cells appear after the cornea has been in the stain for about an hour. Similar cells occur in the conjunctiva. The cells can also be stained with toluidine blue, or with brilliant cresyl blue, used in the same way as the methylene blue; they appear after about 3 hr. staining. Occasionally, but not always, the basal layer branched cells can be seen in fresh corneae examined by phase contrast microscopy. In electron micrographs, the large mitochondria, well-developed endoplasmic reticulum, and apparent lack of any attachment plaques, are points of resemblance with the dendritic cells of human epidermis described by Clark & Hibbs (1958); a point of difference is that the cell outlines of the corneal branched cells do not appear noticeably smooth, rather the reverse. In microscopical preparations also the corneal branched cells appear more angular and wiry than epidermal dendritic cells. Billingham & Medawar (1953) argued, but did not profess to demonstrate conclusively, that the high level dendritic cells of mammalian epidermis are effete, desquamating melanocytes; their argument was based partly on the presumed absence of any form of branched cell from the corneal epithelium and from other epithelia lacking melanocytes. Silvers (1957) and Charles & Ingram (1959) think that the high level dendritic cells are not related to melanocytes. Zwillenberg (1958) has shown that there are high level dendritic cells, with counterparts at deeper levels, in oesophageal and vaginal epithelia. It may be that the branched cells in the cornea have no homology with those in other epithelia: the fact that branched cells in skin, cornea, and other stratified squamous epithelia, can all be stained with quinone-imine dyes, means no more than that their oxidative-reductive reactions differ from those of the epithelial cells surrounding them; it does not in itself argue that the cells are homologous with one another. In skin and in cornea, the appearance of the branched cells in electron micrographs also indicates that they are physiologically differentiated from the epithelial cells (but it should be noted that the dendritic cells described by the authors cited are melanocytes rather than high level dendritic cells, even though some of them may not contain melanin). Although it has not been shown that they are homologous, the presence of branched cells in various epithelia does suggest that they must have a function; in the cornea, at least, the idea that they act as Schwann cells can be excluded. An obvious hypothesis is that they are concerned with the transport of metabolites within the tissue; if this is so, epidermal melanocytes may perhaps be regarded as a special case of dendritic cell, concerned with the transport of melanin precursors. Various authors have suggested that melanocytes, or Langerhans cells, may have nutritive, or conductive, functions; references will be found in Billingham (1948). Many of these suggestions, however, depended on the idea that processes from dendritic cells linked the epithelium to the underlying corium, which is not so, although melanocytes may underlie the epidermis. In the cornea, basal layer branched cells were never seen to have any contact with the stroma; if then they do have a 'conductive' function, it can be within the tissue only. The idea that basal layer branched cells are effete cellular elements from elsewhere is not attractive, as in such a case they would presumably have been derived from the stroma, and it would be expected that they would occasionally be seen passing from the stroma to the epithelium; this has not been seen. It remains possible that the cells could have migrated from the limbus, within the epithelium.

It will be seen from Text-fig. 3 that the branches of the basal layer branched cells may be both long and thin; they are sometimes no more than 0·75 μ in diameter. This is also the diameter of the large epithelial nerve fibres, so that there can be confusion. In most cases the appearance of the cytoplasm and of the edge of the cell does allow differentiation; the outline of a basal layer branched cell is less smooth than that of a nerve fibre, and often more widely separated from the surrounding epithelial cells. In longitudinal section, the mitochondria in a nerve fibre are more elongated, and there are more of them, than in the arms of a basal layer branched cell. There remained a number of cases where there was doubt, and for this reason the number of coarse fibres recorded from the epithelium was probably less than the true number. The confusion did not arise in the case of the smaller nerve fibres, but they might easily be missed because of their size.

PASSAGE OF NERVES FROM THE STROMA TO THE EPITHELIUM

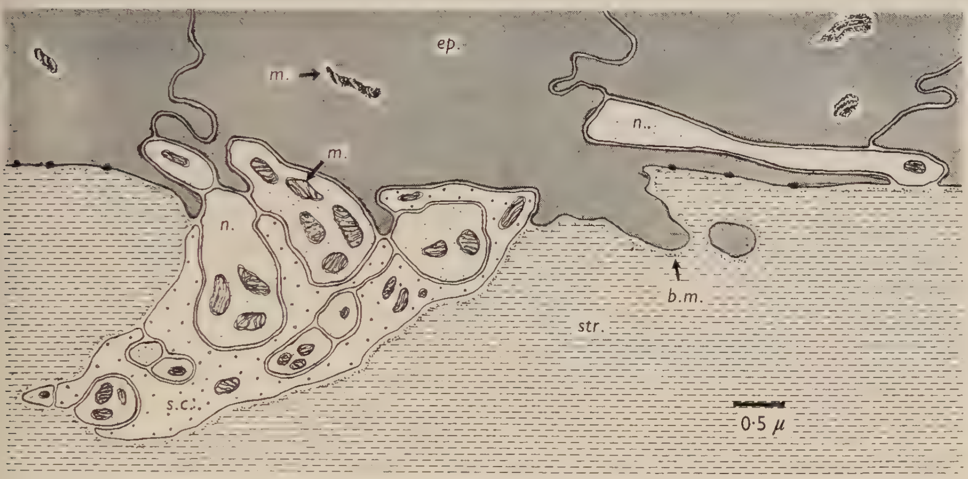
Bundles of nerve fibres pass from the stroma to the epithelium at scattered points over the whole extent of the cornea. Finding the point of entry, for electron microscopy, was a matter of chance, as the position could not be predicted and most series of sections did not include one; less than a dozen such points were seen in the course of the investigation.

Both coarse and fine nerve fibres enter the epithelium. They may be branches from fibres running in the subepithelial plexus, or an entire bundle of that plexus may rise into the epithelium. Pl. 3, fig. 21 shows branches of coarse fibres of a subepithelial bundle passing into the epithelium, as seen when the bundle is cut longitudinally. Where the fibres approach the epithelium, processes from the basal layer cells often reach down into the stroma; there is such a process to the right of the nerve in Pl. 3, fig. 21, but the scale is too small to show it clearly. It is more obvious in Text-fig. 4: this is a diagrammatic reconstruction from four serial sections of micrographs taken originally at a magnification of 15,000 times. The scale given is only approximate. It was not clear from the sections available whether the fibre on the right, which is cut lengthwise, was part of this bundle, or whether it had entered the epithelium with another bundle not far away.

In the larger bundles, like that of Text-fig. 4, the nerve fibres are enclosed either in Schwann cells or in epithelial cells throughout their passage into the epithelium, but in small bundles of fine fibres the Schwann cell may not cover all the fibres. Pl. 3, figs. 22–24 show a bundle of three fine fibres in the epithelium and in the stroma. For some distance from the epithelium there was not much Schwann cytoplasm in the nerve bundle; in Pl. 3, fig. 24 two small lobules of Schwann cell cover the middle nerve fibre but not the two lateral ones. These three fibres were followed in serial sections until they joined another bundle of the subepithelial plexus, part of which is shown, incidentally, in Pl. 4, fig. 28.

Most of the nerve fibres indent the basal epithelial cells to a distance of about $\frac{1}{2}\ \mu$, but many are seen in shallower grooves which are not closed off from the basement membrane of the epithelium; there is no significance in the depth of enclosure. Single fibres or leashes may become invaginated in a closed groove which later becomes open. Similarly, fibres may be enclosed within an epithelial cell, or between adjacent cells; it is simply a matter of position.

In methylene blue preparations, the epithelial fibres may be varicose. Varicosities were also found in the osmic-fixed electron microscope material. A varicosity might occur where a fibre branched, or might be at a point where there was no branching but where several mitochondria occurred together. In those parts of a fibre between varicosities, mitochondria usually occurred only one at a time. In Pl. 3, fig. 23, two of the fibres are cut at varicosities, as may be seen by comparing this section with the section of the same fibres in Pl. 3, fig. 22.



Text-fig. 4. Diagram to show the entry of a nerve bundle into the epithelium. The different tissues are indicated conventionally. Abbreviations as in the plates.

NERVE FIBRES IN THE EPITHELIUM ABOVE THE BASAL LAYER

At intervals the basal layer nerve fibres send branches into the upper layers of the epithelium. In microscopical preparations the coarser fibres can be seen to ascend vertically through the basal layer, and to divide into two or more branches which form a sparse arborization in the middle layers. The lengths of the branches vary: frequently they extend horizontally between the middle and the upper layers for about 50 μ, but they do not always remain at the same level. Occasionally, fibres penetrate almost to the surface of the cornea, but the majority end in the lower layers of flattened cells. In methylene blue preparations of mouse cornea, only the coarse fibres showed this striking tree-like arborization, which seems to be a general rule, for Zander & Weddell (1951) also state that the finer fibres are more obliquely disposed.

For electron microscopy, high-level epithelial fibres were not at all easily found. It was necessary to search in tangential sections of specimens which had been stained with PTA more heavily than those used in the rest of the investigation. In the tangential sections, vertically disposed fibres would be cut more or less transversely.

In methylene blue preparations, the coarse epithelial fibres appear beaded; there are varicosities where the fibres divide, and along the horizontal branches especially near their ends. Some authors have considered that the beads are artefacts (Rodger, 1951); Zander & Weddell state that beads are present immediately after removal of the cornea, but become more pronounced later. The presence of varicosities has been confirmed in electron micrographs. The material was fixed in osmium tetroxide at most a few minutes after removal from the animal, so that it seems unlikely that the beads could be artefacts. Pl. 3, fig. 25 shows a section of a coarse fibre in the lower flattened layers, at a point where it is swollen. This is not actually a terminal varicosity, for portions of unswollen fibre were seen on each side of it (one shows in the figure), but it must be near the end of the fibre. This nerve was followed in serial sections from the deeper layers of the epithelium, but was not found in sections of the same area just above this level. For most of its course the fibre was about 0·75 μ in diameter, but was swollen to about 1·0 μ where it branched above the basal layer, and to more than 1·5 μ in the varicosity illustrated. This fibre was the most superficial that was definitely identified as such in the electron micrographs. A portion of a more proximal region of the same fibre, where it is running horizontally in the middle layers, is shown in Pl. 4, fig. 34.

Fine fibres were encountered in the middle layers of the epithelium more often than the coarse ones, but were difficult to follow in serial sections. The smallest fibres seen were 0·25 μ in diameter. Typically the fine rising fibres run between the epithelial cells, but some are enclosed in a groove as in the basal layer. Pl. 3, fig. 26 shows a fibre between two middle layer cells, and Pl. 3, fig. 27 a fibre deeper in the epithelium, which is wrapped in the side of a basal layer cell.

SUBMICROSCOPIC STRUCTURE OF THE NERVE FIBRES

There are several descriptions of the fine structure of axoplasm in the literature. For instance, Vial (1958) distinguished neurofilaments with diameters ranging from 100 to 200 Å., endoplasmic reticulum appearing as elongated vesicles 250–500 Å. across with their long axes parallel to the axis of the nerve fibre, mitochondria, and various particulate components.

Mitochondria are obvious features of most of the illustrations of corneal nerve fibres presented here; they are not in any way remarkable except that they are more likely to be concentrated in the varicosities of fibres than elsewhere.

The neurofilaments of the corneal nerve fibres illustrated can be seen to be of two kinds. There may be fine solid filaments which in transverse section look like dark dots 100–150 Å. in diameter, or less definite filaments 200–250 Å. in diameter, which in some micrographs appear to be tubular. The small dense filaments occur in greater numbers in the more proximal parts of the nerve fibres, the larger, vaguer filaments are more common distally; there is, however, no sharp transition. Fibres such as those shown in Pl. 1, fig. 11 or Pl. 2, figs. 14 and 15 show numbers of the smaller filaments, though larger ones are present also; the subepithelial fibres of Pl. 2, fig. 13 have the larger type only. In Pl. 4, fig. 28 are two nerve fibres, one with small and one with large filaments. In the epithelium, the fibre of Pl. 2, fig. 17 shows the small type, those of Pl. 2, fig. 18, the large. In Pl. 4, fig. 29 is a leash of fibres in the basal layer of the epithelium, which have the larger type of filament, although the two fibres indicated by arrows also contain a number of small solid filaments; the serial sections of this leash showed the same appearance. In nerve fibres cut longitudinally the small solid filaments show clearly, as in Pl. 4, fig. 30, which is part of the subepithelial bundle of Pl. 3, fig. 11, though in a different section. Pl. 4, fig. 31 shows the small type of filament in a nerve fibre in the basal layer, cut lengthwise. Pl. 4, fig. 32 is a rather indistinct picture from a tangential section of two basal layer fibres with the larger type of filament; arrows indicate the elongated filaments; at the right of the picture the fibres are bending and are not cut longitudinally. Above the basal layer of the epithelium, the small solid filaments which are so conspicuous in most of the stromal fibres were not seen.

The observation that the small solid neurofilaments of the sensory nerve fibres in the cornea do not extend to the nerve endings but are replaced in the more distal parts of the fibres by thicker, less dense filaments, is of particular interest in view of the difference in the submicroscopic organization of dendrites and axons in the cerebral cortex, reported by Gray (1958). The dendrites contain tubules about 230 Å. in diameter, while axons contain solid filaments about 100 Å. across, though there may also be a few tubules in the axons. Dilly (1959) has confirmed these observations in the medulla of goldfish. In electron micrographs of the cornea the tubular structure of the 200 Å. filaments is never as clear as it is in similar pictures of the central nervous system, but it does appear in a sufficient number of cases to make it probable that the larger filaments are in fact hollow. Pl. 4, fig. 33, shows an enlargement of part of a basal layer fibre with the tubular appearance. Gray (1959) discusses the technical reasons why small tubules of this sort may be difficult to make out in electron micrographs; comparison of the dendritic tubules, illustrated in Gray's 1959 paper, with those in the corneal nerves, will show how similar they are. The smaller solid filaments look exactly alike in the two situations.

In the basal layer nerves the tubular filaments run parallel with the axes of the fibres, but in the rest of the epithelial nerves this organization is less marked. Pl. 4, fig. 34 shows part of a nerve which is running horizontally in the middle layers; it is the same fibre which appears in Pl. 3, fig. 25, but cut lengthwise at a more proximal position. There are traces of a longitudinal organization in the cytoplasm, but it is not very obvious. Pl. 4, fig. 35, is a photographic enlargement of part of the same micrograph, to show tubular filaments. In the varicosity at the end of the fibre (Pl. 3, fig. 25) it is impossible to distinguish the filaments as such, though dots of the right size are present.

Vesicular profiles about 300–400 Å. across can be found in many of the corneal fibres; there are several in Pl. 4, fig. 30, and two which are especially conspicuous just within the lower cell membrane of the large fibre of Pl. 2, fig. 14. Presumably such profiles represent part of the endoplasmic reticulum. Similar 'vacuoles', and some profiles which look like portions of channels, are present in the varicosity in Pl. 3, fig. 25. Channels or vacuoles seemed to be more common here than they had been in the more proximal part of the nerve, but there was nothing like the accumulation of 'vesicles' seen by Wersäll (1956) at the ends of some sensory fibres of the auditory nerve. Vesicles described by Pease & Quilliam (1957) in the nerves of Pacinian corpuscles or by Pease & Pallie (1959) in cutaneous nerves were much more like those seen in the corneal nerves. The vacuolar profiles of over 300 Å. in diameter are distinct from the tubular filaments described above, whose calibre never exceeds 250 Å., and from the finer solid neurofilaments.

It would be premature to speculate about the possible significance of the distribution of the small solid and larger tubular filaments in the corneal nerves, as no information is available about other peripheral nerves which is comparable. All published electronmicrographs of osmic-fixed neurofilaments are taken from specimens embedded in methacrylate; the estimated thickness of the filaments varies from 60 Å. (Elfvin, 1958) to 200 Å. (Vial, 1958). In methacrylate material of the corneal nerves, some tubular profiles of the right size were seen in basal layer nerves, but there was not the characteristic spotted appearance of transverse sections of these nerves in araldite. In the stromal nerves, the dark dots which are the most noticeable feature of transverse sections of fibres in araldite material, did not appear at all in methacrylate material. It is tempting to compare the distal parts of the corneal sensory nerve fibres, with tubular filaments, to dendrites of the central nervous system, and to suppose that they are characteristic of that part of the sensory nerve fibre which is specialized for the reception of stimuli, but it would be very rash to do so unless information were available about other peripheral nerves, both afferent and efferent, which had been fixed in osmium tetroxide and embedded in araldite.

DIAMETERS OF CORNEAL NERVE FIBRES

As most of the nerve fibres in the cornea are too slender to allow accurate measurement of their diameters by light microscopy, it was hoped that the present study would allow an analysis of fibre diameters. For reasons explained below, it proved impracticable to construct an accurate fibre spectrum, though measurements made on a few representative nerves are given. All measurements were made on enlarged prints of electron micrographs of araldite sections and are presented with no attempt at correction, although, as the fibres were measured in a direction perpendicular to the surface of the cornea, which was also the direction of cutting, compression may have caused the figures to be too low. There is however no reason to distrust the relative measurements of the fibres.

The diameters of the largest fibres seen in the stromal nerve bundles were 1·5 μ in the subepithelial plexus, 2 μ in the mid-stromal plexus, and just over 2 μ in the deep peripheral bundles (medullated fibres were measured inside the myelin). The smallest fibres seen at any of these levels measured 0·15 μ. When it was possible to follow individual fibres of a bundle in serial sections over some distance, it appeared that the range of fibre size in any one section was deceptive. Even when a fibre could not be said to be beaded, there might be a difference of 0·25 μ between the greatest and the least measurements of its diameter, in different sections. The variation was not due to differences of compression in different sections, for where one fibre was thin, an adjacent fibre was likely to be thick, and vice versa. As it was only rarely possible to follow individual fibres through a long series and so find the average diameter, the attempt to make an analysis of fibre size was abandoned.

Text-figs. 5–8 are representations of the diameters of nerve fibres found at various levels of the stroma. The bundle of Text-fig. 5 is not complete, being too large to fit into the sections; part of it appears in Pl. 1, fig. 11. It is possible that branches of some of these fibres also appear in Text-fig. 6. In Text-fig. 7, the bundle of *b* is that illustrated in Pl. 2, fig. 15; the bundle of 7*a* was small and rounded. Part of 8*b* is shown in Pl. 2, fig. 14, while 8*a* is the bundle of Pl. 3, fig. 21, measured where it was cut transversely.

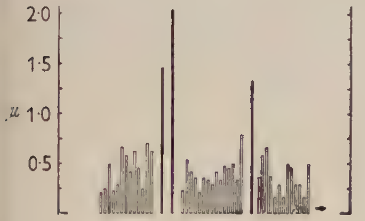


Fig. 5

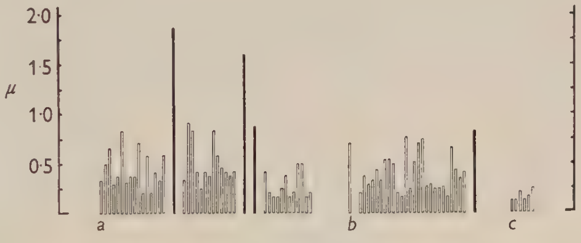


Fig. 6

Text-fig. 5. Deep peripheral bundle. The height of each column represents the mean diameter of an individual fibre, measured at a total magnification of 24,000 times. Black columns myelinated fibres, unshaded columns unmyelinated fibres arranged in groups as they occurred within Schwann cells.

Text-fig. 6. Bundles of the mid-stromal plexus, in the same quadrant of a cornea as that of Text-fig. 5. Convention as in Text-fig. 5.

In text-figs. 7 and 8, the columns which are set close together and linked at the base represent branches of a single fibre. Where a fibre branched, in the stroma or in the epithelium, as it was followed in serial sections it would become thicker and might show an indentation of the cell membrane (as in Pl. 2, fig. 18); then two fibres would appear where one had been before; there seemed to be no specialization at the point of branching. In the stroma division occurred within the territory of a single Schwann cell. The branches were not necessarily of the same size. The greatest increase in the diameter of a fibre occurred where it was about to branch, while the greatest decrease occurred where a fibre was crossing over an adjacent one as they interlaced within a bundle. In the epithelium the fibres are actually beaded, so that the variation in the diameter of an individual fibre may be even greater than in the stroma. The largest fibres seen in the basal layer of the epithelium had maximum diameters of 1 μ, though beads at higher levels may exceed this.

The variation in the diameter of individual fibres makes it difficult to distinguish categories of fibres. Those nerves which enter the cornea as medullated fibres form a distinct class; they are known to send branches to the epithelium, which are probably those forming tree-like arborizations, like the fibre of Pl. 3, fig. 25 and

Pl. 4, fig. 34. The larger fibres of the subepithelial plexus, of diameter around 1 μ, are probably branches of the myelinated fibres. The smallest unmyelinated fibres, which often run in small bundles like that of Pl. 2, fig. 13, probably constitute another class, for they were seen at all levels in the cornea. The diameters of such fibres varied between 0·15 and 0·5 μ, with a mean near 0·25 μ. There remains a possible third class of fibres, those which at the deeper levels of the stroma are unmyelinated, with diameters of from 0·5 to 0·75 μ. Even in the deep peripheral bundles it is possible (although it seems unlikely) that these fibres are the branches of fibres which were previously medullated, while reference to text-figs. 7*b* and 8*a* will show that in the subepithelial plexus it becomes impossible to distinguish fibres of this category. Nevertheless, when viewing the material as a whole, there was a subjective impression that, in the deeper levels of the cornea, a distinction could be drawn between fibres of mean diameter less than 0·5 μ, and those whose mean diameter was somewhat larger.

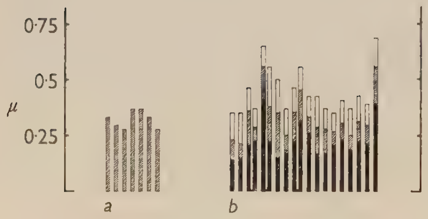


Fig. 7

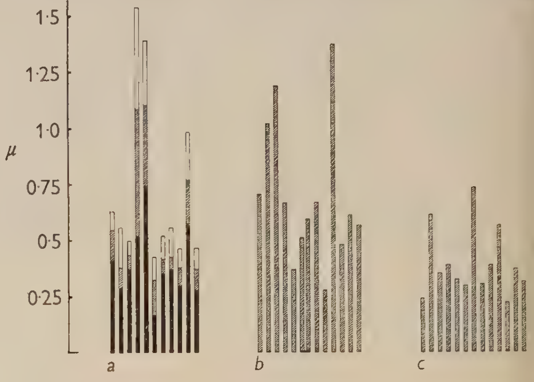


Fig. 8

Text-fig. 7. Two bundles of the deeper part of the subepithelial plexus. The height from the base line of each cross-hatched column gives the mean diameter of a fibre; where enough serial sections were available, the maximum and minimum diameters encountered are also indicated, by the heights of the white and black parts of the columns, respectively. The larger fibres in *b* were traced in twenty-four sections. Measurements made at a total magnification of 54,000 times.

Text-fig. 8. Three subepithelial bundles, *a* from nine sections, *b* and *c* each from three. Convention and magnification as in Text-fig. 7.

DISCUSSION

Various authors have made a distinction between myelinated and unmyelinated nerve fibres supplying the cornea, and some have considered that the latter are autonomic fibres; references to the literature are given by Rodger (1953). As a result of his experiments Rodger concluded that all the nerve fibres supplying the cornea (in cats) originated from the ophthalmic division of the trigeminal nerve. Rodger inclined to the opinion that there is no true morphological distinction between coarse and fine fibres in the stroma, the coarse fibres being merely those which have retained the myelin sheath longer than the others. The present evidence from electron micrographs confirms what can, indeed, be seen by light microscopy, that the fine unmyelinated fibres enter the cornea alongside the coarser myelinated ones; this does not, of course, mean that the unmyelinated fibres are autonomic in origin.

Lele & Weddell (1956) have shown that the cornea is sensitive to thermal and tactile stimulations as well as to those of pain. They state that the unencapsulated, or free, nerve endings of the cornea cannot be distinguished from one another on morphological grounds, though they are well aware that different fibres may end at different levels in the cornea. Even though all the nerve fibres in the cornea come to free endings, it can scarcely be said that there is no morphological distinction between fibres. There is, for instance, a distinction between thick fibres which enter the cornea with a myelin sheath, and thin fibres which are unmedullated throughout their course. There can be a distinction between fibres entering the epithelium, and fibres ending in the stroma. In the epithelium, there can be a distinction between the thick fibres with a tree-like arborization (a vertical trunk and more or less horizontal branches) and the thinner fibres which, though they may branch, do so in a less startling manner. At the submicroscopical level, furthermore, it may be possible to distinguish between those fibres which replace the solid 100 Å. neurofilaments by tubular 200 Å. filaments before they enter the epithelium, and those which retain the finer neurofilaments for at least part of their course in the basal layer. Whether there is any functional significance in any or all of these morphological distinctions is another question; even if there is, it certainly does not imply that any one, single, fibre need be competent to transmit a particular sense modality, as the application of von Frey's theory of specific irritability, in its extreme sense, might suggest. For a discussion of the 'specific fibre' and the 'pattern' theories of cutaneous sensation, reference should be made to Sinclair (1955), and to Lele & Weddell (1956).

A possibility which must be considered is that of interaction between adjacent fibres. Considering unmedullated fibres in the dorsal roots, Gasser (1955) thought it unlikely that there is any cross-excitation between the axons; in the dorsal root nerves, several fibres may be enclosed in a Schwann cell, although they are individually wrapped, but the same fibres do not remain parallel over any distance, and pass into different Schwann cells as they interlace. In the stromal nerve bundles, nerve fibres may be at times actually in apposition, and therefore in a closer relationship to each other than in the dorsal root nerves; such fibres may be branches of the same fibre, but are not always so; the same is true in the leashes of the basal layer of the epithelium. In this respect, the corneal unmyelinated fibres are intermediate between those of the nerve trunks and those of the olfactory nerves (see Gasser, 1958). Nevertheless, in the corneal bundles as in those of the nerve trunks the fibres interlace, so that any two fibres will not necessarily remain adjacent for more than a short distance.

It is usually assumed that, except in epithelia, peripheral nerve fibres are everywhere enclosed by Schwann, or satellite, cells (see, for instance, Schmitt, 1958). There are exceptions to this rule; in the organ of Corti in the ear, Smith & Dempsey (1957) and Engström & Wersäll (1958) found that some nerve fibres run completely naked for a short distance, after they lose the myelin sheath. In the upper levels of the corneal stroma, though it was not established that nerve fibres are ever completely devoid of an accompanying Schwann cell, it was quite common for considerable areas of nerve cell membrane to be in contact with the collagenous *substantia propria* (except for the intervention of a 'basement membrane', which was not universally present). Within the epithelium, the epithelial cells are in as close a relationship to the nerve fibres as are the Schwann cells in nerve bundles, and may therefore perform whatever supporting or sustaining functions are normally the duty of the Schwann cells.

SUMMARY

Electron micrographs are presented which bring out certain aspects of the fine structure of corneal tissues, not hitherto illustrated. These include the form of attachment plaques and mitochondria in the epithelium, and the form and interrelationships of the stromal cells. It is suggested that certain channels, bordered by stromal cells, near the periphery of the cornea, may be concerned with the movement of fluids in the cornea.

The submicroscopic anatomy of nerves at various levels in the stroma, and in the epithelium, is described. In the subepithelial plexus of the stroma, nerve fibres are accompanied by, but not necessarily wholly enclosed in, Schwann cells. Schwann cells do not penetrate the epithelium, but as nerve fibres enter the epithelium they become enclosed in grooves in the epithelial cells. It is shown that those epithelial cells of the type here called 'basal layer branched cells' do not have any particular relationship to nerve fibres.

The course of nerve fibres in and above the basal layer of the epithelium is traced, and the occurrence of beading on some of the fibres is confirmed; beads are often associated with accumulations of mitochondria.

Two kinds of neurofilaments can be distinguished in the nerve fibres; there are small solid filaments about 100 Å. in diameter, which are characteristic of fibres in the stroma, and less definite filaments about 200 Å. in diameter, and apparently tubular, which are characteristic of the more distal parts of the fibres. As a nerve fibre approaches its ending, the axoplasm becomes less filamentous and contains more vacuolar profiles, but there is no striking accumulation of 'synaptic vesicles'.

Measurements of the diameters of nerve fibres at various levels in the cornea are given, but, owing to the variation in the calibre of individual fibres, an accurate spectrum of fibre diameter is not attempted.

I should like to express my gratitude to Prof. J. Z. Young for the use of the facilities of his laboratory, and especially to Dr J. D. Robertson for instruction in electron microscopy.

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EXPLANATION OF PLATES

Key to abbreviations used in plates

b.l.b.c.	basal layer branched cell.	n.	nerve fibre.
b.m.	basement membrane.	nuc.	nucleus.
coll.	collagen fibrils.	s.c.	Schwann cell.
d.	attachment plaque.	str.	stroma.
ep.	epithelial cell.	str.c.	stromal cell.
m.	mitochondrion.		

All figures are electron micrographs of ultra-thin sections of mouse corneae fixed in osmium tetroxide and stained with phosphotungstic acid. All except Pl. 2, fig. 19 are embedded in araldite. The scale on each figure measures 0·5 μ, unless otherwise stated.

Plate 1

Fig. 1. Boundary between two middle layer epithelial cells, with attachment plaques (arrowed).

Fig. 2. As Fig. 1, at a greater magnification. The arrows indicate the cell membranes.

Fig. 3. Base of the epithelium showing attachment plaques, basement membrane, and three nerve fibres. Latitudinal section.

Fig. 4. Mitochondrion in a middle layer epithelial cell.

Fig. 5. Mitochondria in a superficial cell of the flattened layers. External membrane arrowed.

Fig. 6. Vertical section of part of a stromal cell, showing the endoplasmic reticulum. The nucleus would be to the left, the flattened extension to the right.

Fig. 7. Vertical section of the flattened extensions of two stromal cells.

Fig. 8. Transverse section of a fine process from a stromal cell (arrowed).

Fig. 9. Vertical section of two stromal cells near the periphery of the cornea, with a space between. Longitudinal section.

Fig. 10. Vertical section of part of a channel in the stroma, near the periphery of the cornea. Longitudinal section.

Fig. 11. Part of a transverse section of a deep peripheral nerve bundle, showing the external sheath intruding between two Schwann cells. Myelinated fibre on the left, group of unmyelinated fibres and Schwann cell nucleus on the right. Longitudinal section.

Fig. 12. Transverse section of a subepithelial nerve bundle near the periphery of the cornea. Longitudinal section.

Plate 2

Fig. 13. Transverse section of a small nerve bundle of the subepithelial plexus.

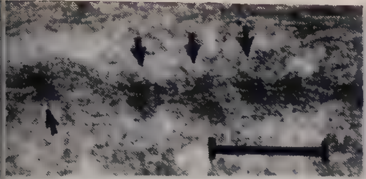
Fig. 14. Transverse section of part of a large flattened nerve bundle of the subepithelial plexus.

Fig. 15. Transverse section of a nerve bundle of the subepithelial plexus, deeper than that of Fig. 14. Only a few of the nerve fibres are labelled.

Fig. 16. Transverse section of a small nerve bundle of the subepithelial plexus.

Fig. 17. Transverse section of a nerve fibre in the basal layer of the epithelium. The small arrows indicate the cell membrane of the epithelial cell. Latitudinal section.

Fig. 18. Transverse section of a leash of four nerve fibres in the basal layer of the epithelium. The fibre on the right is about to divide. Latitudinal section.



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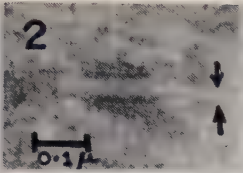

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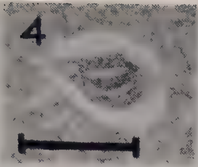

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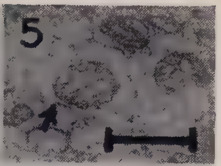

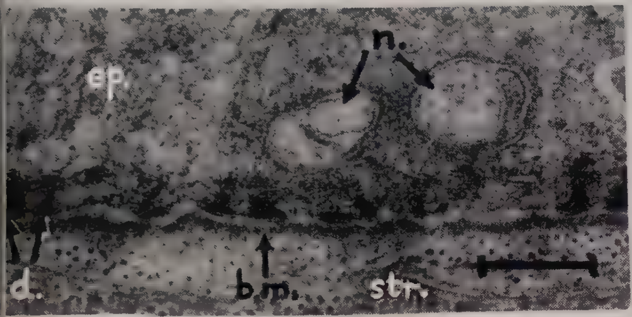

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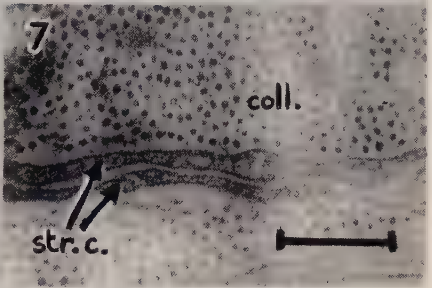

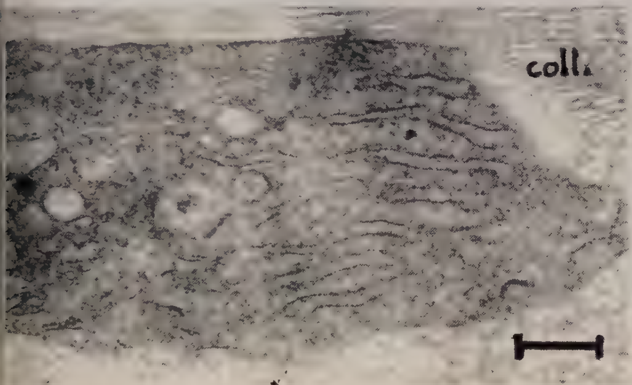

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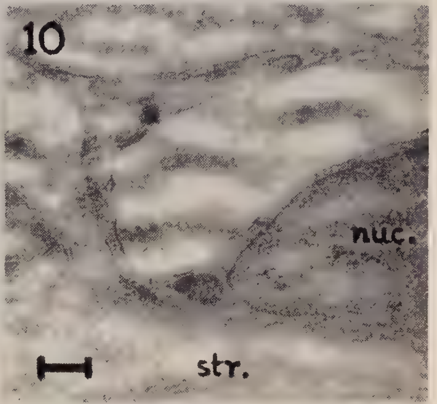

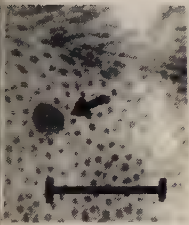

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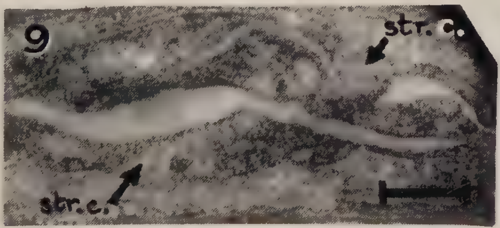

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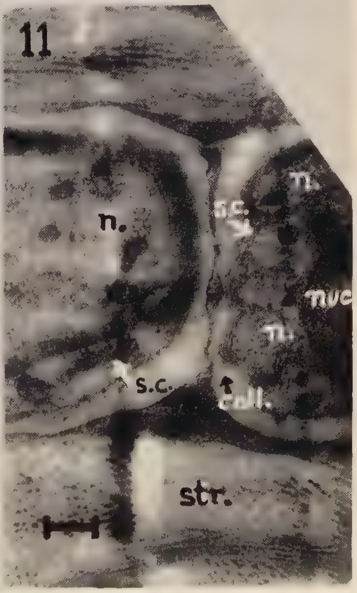
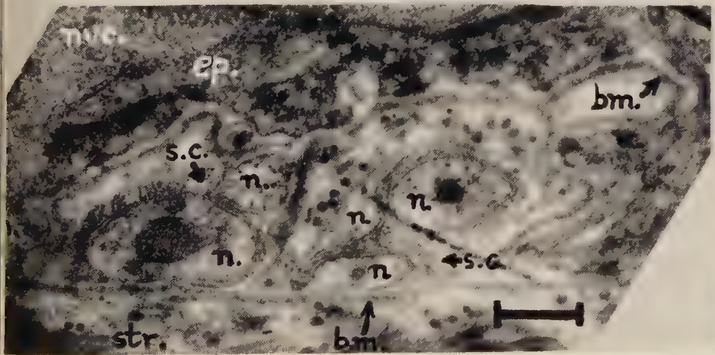
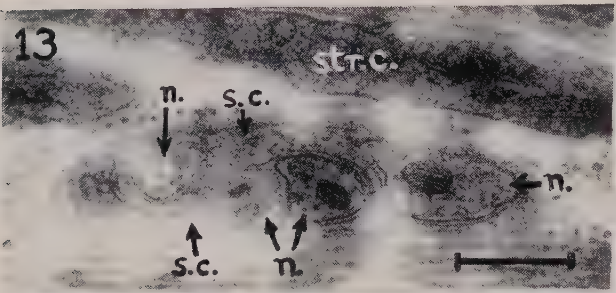
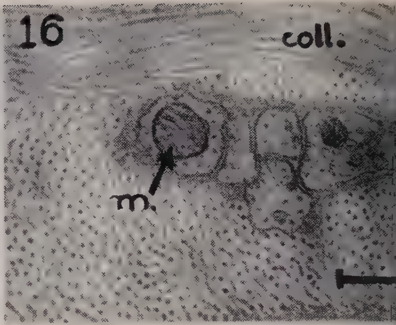
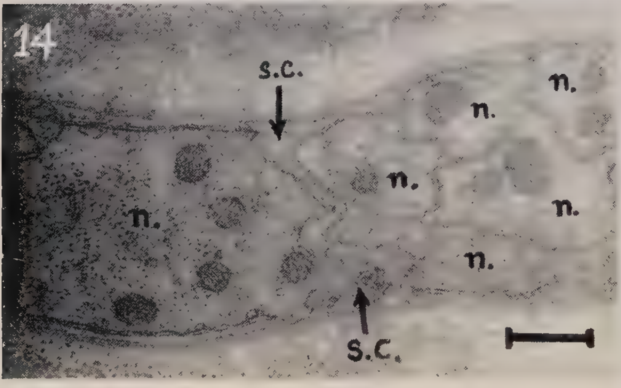


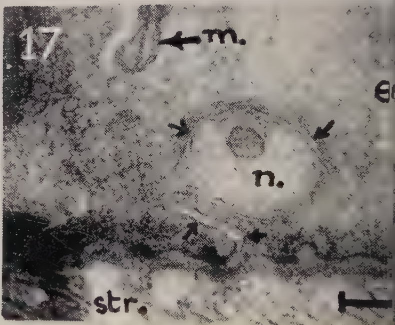


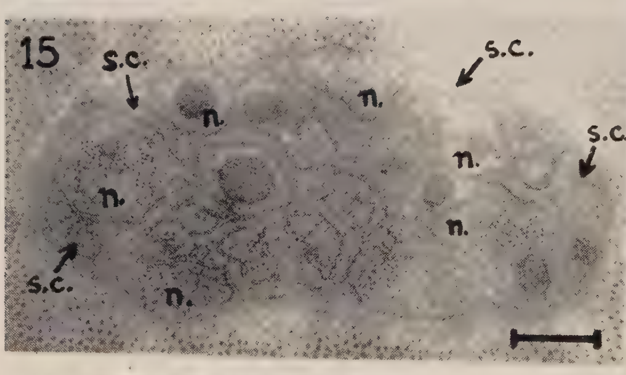
WHITEAR—ELECTRON MICROSCOPE STUDY OF MOUSE CORNEA

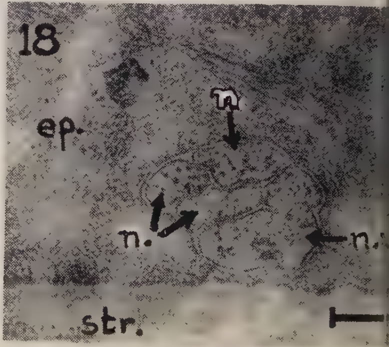


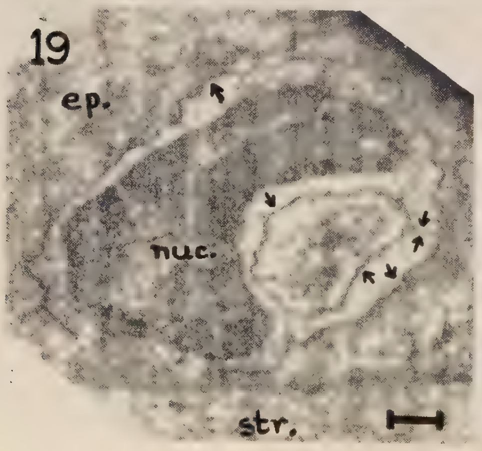


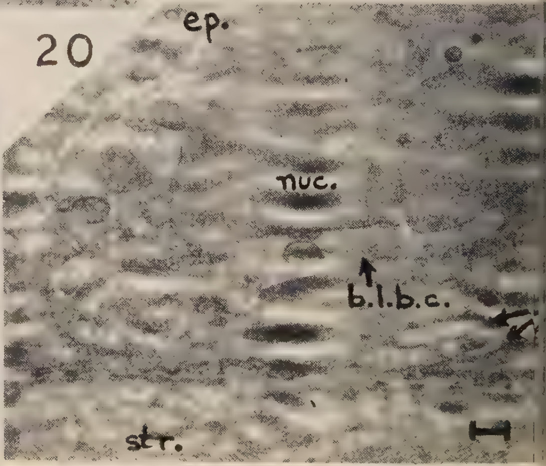












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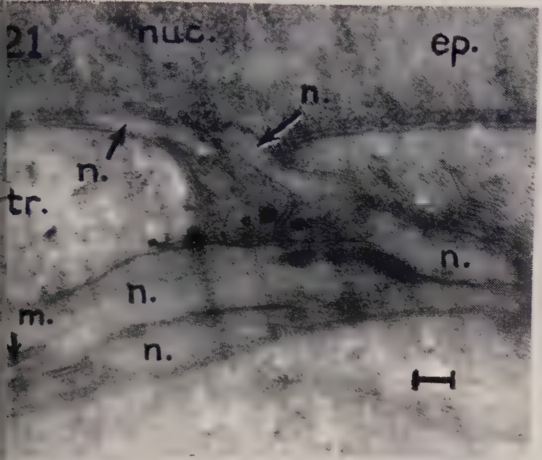
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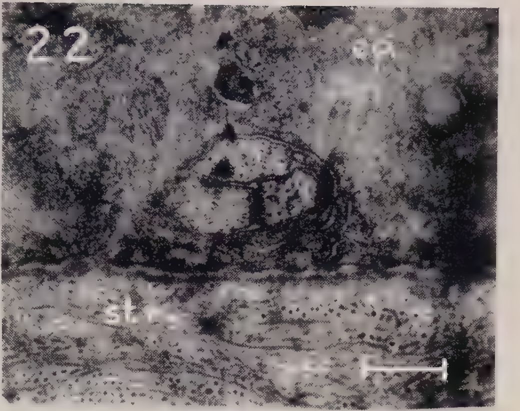
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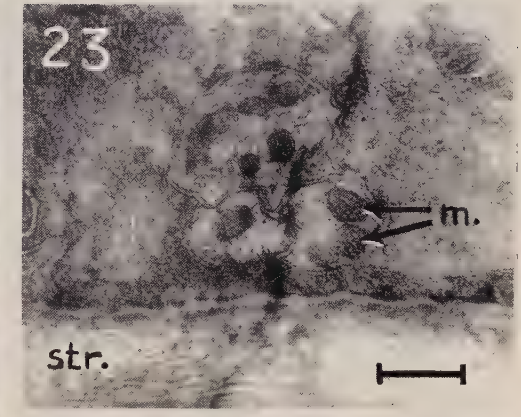
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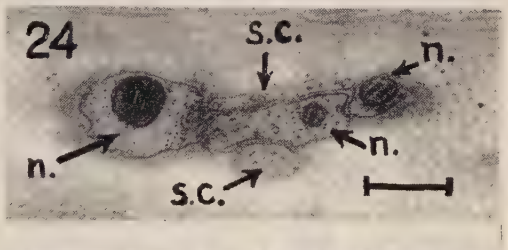
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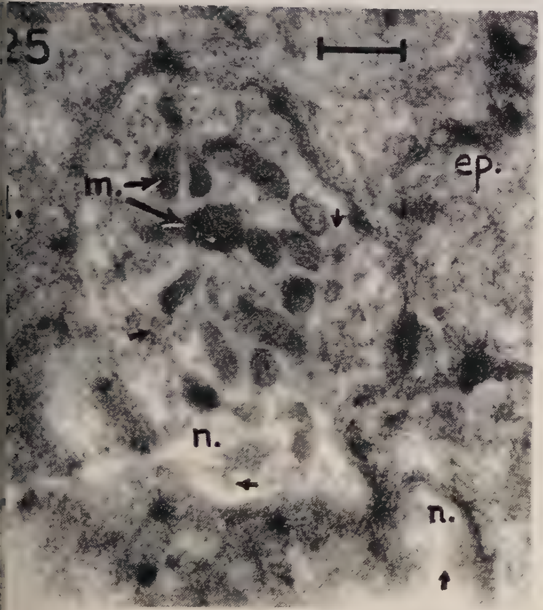
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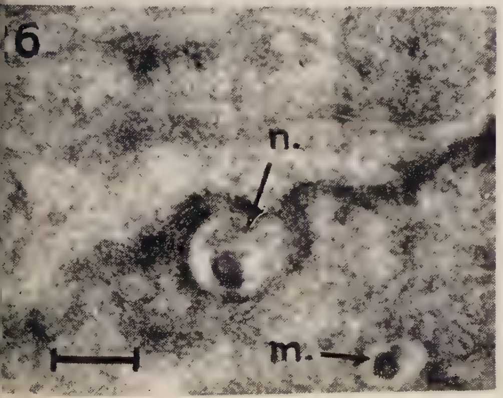
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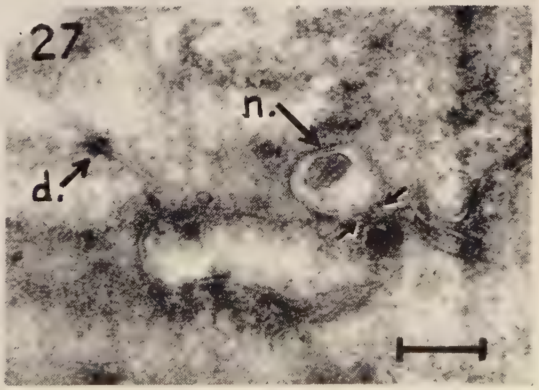
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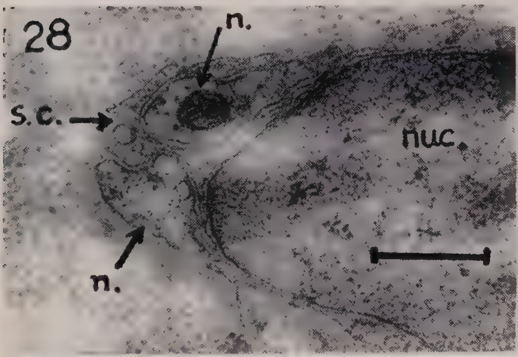

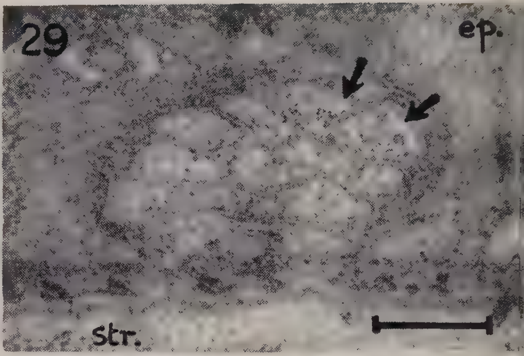

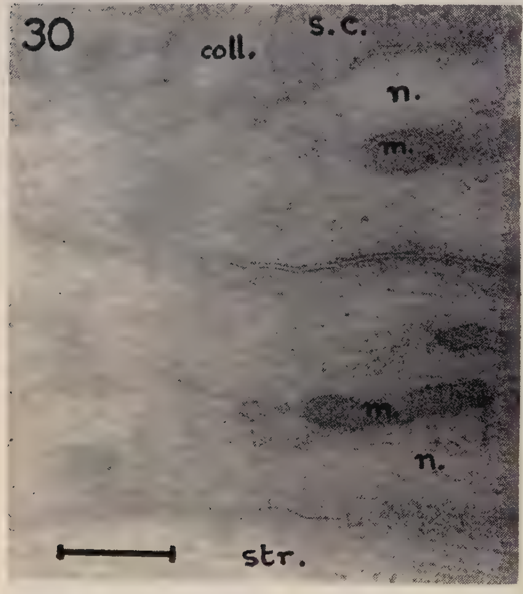

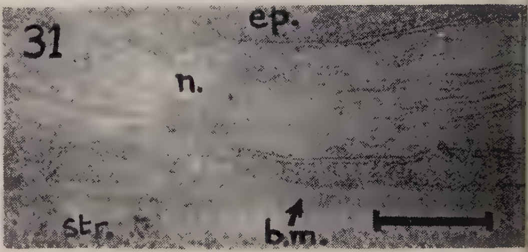

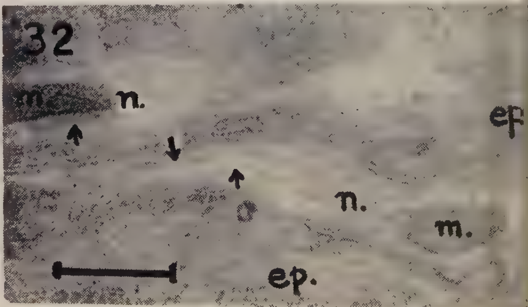

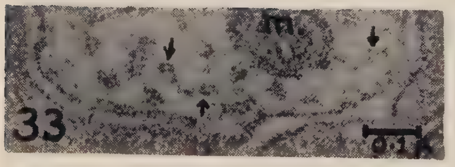

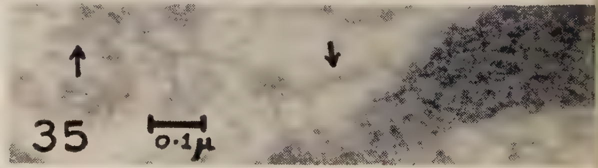

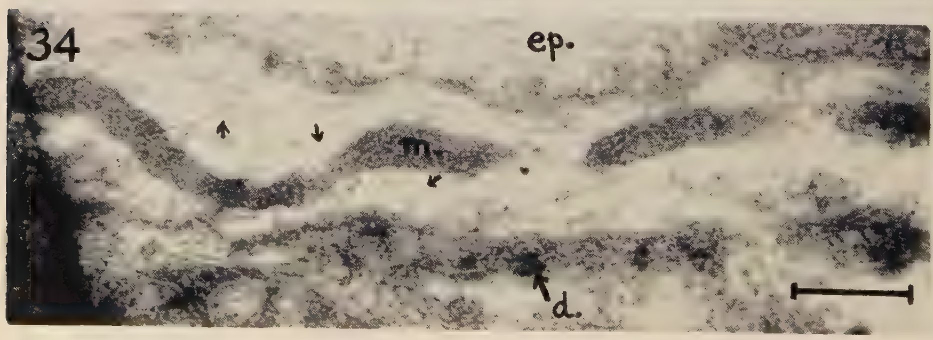

WHITEAR—ELECTRON MICROSCOPE STUDY OF MOUSE CORNEA

Fig. 19. A basal layer branched cell enclosing the arm of another. The small arrows indicate the cell membrane of the first cell.

Fig. 20. A basal layer branched cell, showing nucleus and cytoplasmic contents, and two nerve fibres wrapped in the adjacent epithelial cell.

PLATE 3

Fig. 21. A nerve bundle of the subepithelial plexus cut longitudinally, sending branches into the epithelium.

Fig. 22. Transverse section of a bundle of three nerve fibres in the basal layer of the epithelium. Latitudinal section.

Fig. 23. The fibres of Fig. 22 in a serial section.

Fig. 24. The fibres of Figs. 22 and 23, in a serial section, in the stroma, with Schwann cell cytoplasm.

Fig. 25. High-level epithelial nerve fibre cut at a varicosity. Vesicular profiles supposed to be parts of endoplasmic reticulum indicated by small arrows. An adjacent unswollen part of the same fibre appears in the bottom right-hand corner. Tangential section.

Fig. 26. Transverse section of a nerve fibre between two middle layer epithelial cells. Tangential sections.

Fig. 27. Transverse section of a nerve fibre in the side of a basal layer epithelial cell. The small arrows indicate the cell membrane of the epithelial cell. Tangential section.

PLATE 4

Fig. 28. Transverse section of part of a nerve bundle in the subepithelial plexus, with a Schwann cell and two nerve fibres.

Fig. 29. Transverse section of a leash of small nerve fibres in the basal layer of the epithelium. The arrows indicate fibres with some solid neurofilaments. Latitudinal section.

Fig. 30. Two nerve fibres of a subepithelial bundle cut longitudinally.

Fig. 31. Basal layer nerve fibre cut longitudinally.

Fig. 32. Two basal layer nerve fibres cut longitudinally, in a tangential section. The arrows indicate 200 Å. filaments.

Fig. 33. Transverse section of a basal layer nerve fibre, with tubular filaments. Latitudinal section.

Fig. 34. Longitudinal section of part of a horizontal branch of a nerve fibre in the middle layers of the epithelium. The small arrows indicate tubular filaments. Tangential section.

Fig. 35. Photographic enlargement of part of Fig. 34; the arrows correspond.

THE LOCALIZATION WITHIN THE DORSAL MOTOR NUCLEUS OF THE VAGUS OF THE EFFERENT FIBRES TO THE RUMINANT STOMACH

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The concept of localized representation within the central nervous system is now generally accepted, especially somatotopic localization in regions such as the cerebral cortex and cerebellum (Fulton, 1949). Within the cranial nuclei, for example in the oculomotor nucleus, it is known that efferent fibres to different muscles are derived from circumscribed neuronal groups within the individual nuclei (Warwick, 1951, 1953).

To investigate any possible specific topographical arrangements within nuclei most workers have used the anatomical method wherein the efferent nerves are sectioned and the resultant retrogressive degenerative change examined in the central neurones after a suitable time interval. The validity and reliability of this method has been discussed by Molhant (1910), with particular reference to the dorsal motor nucleus, and more recently by Warwick (1951) in connexion with the oculomotor nucleus. Molhant (1910) and Getz & Sirnes (1949) have examined the dorsal motor nucleus of the rabbit with this technique and show definite topographical arrangements. Mohiuddin (1953), on the other hand, using the same technique was unable to demonstrate any precise localization in the dorsal vagal nucleus of cells supplying motor fibres to the stomach of the cat. Mitchell & Warwick (1954) show that section of the vagus at all levels in the rhesus monkey produces chromatolytic change in the dorsal motor nucleus and that the middle part of the nucleus is not concerned entirely with cardiac innervation. Kitchell, Stromberg & Davis (1956), in a brief report, show that the cells supplying fibres to the abdominal organs of ungulates and some non-ungulates are situated principally in the rostral part of the dorsal vagal nucleus.

Laughton (1929) showed, by faradic stimulation of the floor of the fourth ventricle of the cat, that the cephalic end of the dorsal motor nucleus has both motor and inhibitory gastrovagal fibres. Bell & Lawn (1955) have adduced evidence that in the sheep the reticulo-rumen and the oesophagus can be induced to contract on electrical stimulation of the lateral reticular formation of the medulla adjacent to the anterior half of the dorsal vagal nucleus.

In order to pursue further the possible localized representation of the stomach within the medullary nuclei of the sheep and goat, a series of vagal sections were made and the retrograde changes studied. In addition, opportunity was taken to study the effect of vagal section on movements of the reticulum and rumen and to assess its effect on rumination.

METHODS

Animals. Eight crossbred goats, and three crossbred Kent sheep varying in weight from 25 to 50 kg. were used. They were maintained on a ration of 6 lb. of hay and ½ lb. concentrate mixture a day.

Anaesthesia. In all cases of neurectomy, narcosis was induced with thiopentone sodium by injection into the recurrent tarsal vein, a cuffed endotracheal tube was introduced and anaesthesia continued by means of cyclopropane in closed circuit.

Operation. In two cases where the whole vagus was divided, the nerve was exposed and sectioned midway in the neck. In all other cases the vagus or its branches were exposed transthoracically. To facilitate exposure of the posterior mediastinum a portion of the seventh rib was removed. The nerves, intrathoracically divided, were doubly ligated as close as possible to the diaphragm and cut between the ligatures. During the manipulation and preparation of the nerves, apnoea was induced by hyperventilation of the animal with oxygen from the bag of the anaesthetic machine. The neurectomies were always carefully checked post-mortem, the actual site of section being easily recognized because of the neuromata that developed where the nerves had been ligated. The sites of the various neurectomies are listed in column two of Table 1.

Histology. To permit retrograde chromatolytic degeneration to develop in the medullary nuclei, 10–15 days were allowed between the time of operation and the time of euthanasia.

After completion of the post-mortem the head was removed and perfused at 120 mm. Hg pressure via the cannulated carotid arteries, the perfusate escaping from the opened jugular veins. When the saline perfusate ran clear it was replaced with 5 % formol saline. The whole brain was then immersed in 5 % formal saline for a few days before removal of the medulla oblongata, which was sliced into pieces suitable for handling histologically, dehydrated, cleared in toluol and embedded in paraffin. Sections were cut serially at 20μ, every tenth section was mounted and stained with thionin by Nissl's method.

The number of cells in the dorsal motor nucleus showing chromatolysis was estimated as a percentage of the total number of cells present. These estimates were correlated for the different levels of the vagal nuclei and are shown diagrammatically in Table 1.

Recording of intragastric pressure. The animals were fitted with intrarumenal cannula which allowed insertion of balloons into the reticulum and rumen (Phillipson & Innes, 1939). The lightly inflated balloons were connected to water manometers arranged to write on a kymograph.

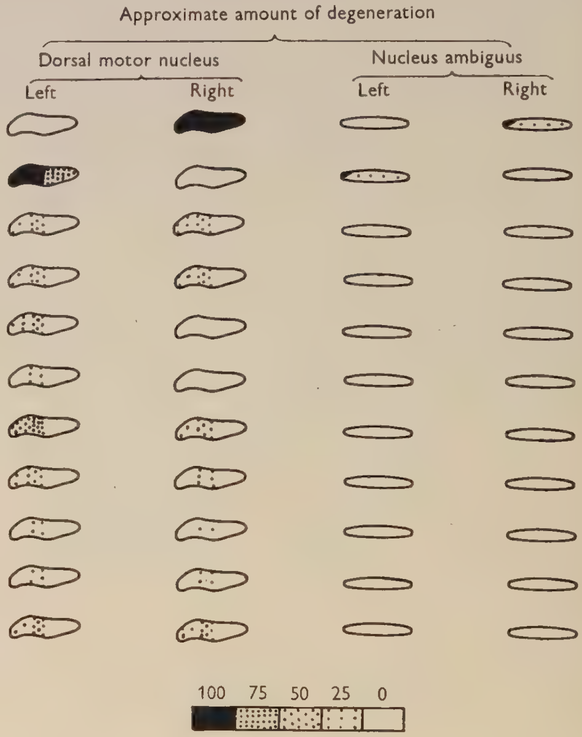
RESULTS

The various neurectomies of the vagus made in the eleven animals is given in Table 1. The distribution of the main vagal trunks in the posterior thorax to form the dorsal and ventral abdominal vagus was found to conform to the description already given by Mangold & Klein (1927). Only in goat 26 was there an anomaly, for in this animal the dorsal abdominal vagus was derived solely from the left vagal trunk.

In all cases, except goat 24, phasic movement of the reticulum and rumen was demonstrated graphically within 48 hr. of nerve section. Goat 24, until the time of destruction, appeared to be in excellent health, continuing to eat normally without the development of any tympanites; but it did not ruminate. Using the method described by Bell & Lawn (1957) for recording rumination over long periods it was shown that in all the animals, except goat 24, normal rumination occurred within 48 hr. and in most cases within 24 hr. Only the two sheep with complete unilateral cervical vagal section showed any tympanites, and in addition these animals had difficulty in swallowing. This apparent interference with the control of the oesophagus subsided within 24 hr. of the operation.

Table 1. *The degree of degeneration in the dorsal motor nucleus of the vagus and nucleus ambiguus following section of the vagus at various levels.*

Animal	Nerve divided
S 1	Right cervical
S 3	Left cervical
S 2	Dorsal abd. vagus
G 22	Dorsal abd. vagus
G 26	Dorsal abd. vagus*
G 28	Left dorsal abd. vagus
G 21	Ventral abd. vagus
G 25	Ventral abd. vagus
G 40	Ventral abd. vagus
G 20	Left ventral abd. vagus
G 24	Both abd. vagi



Approximate percentage of degenerate cells

* **Dorsal abd. vagus derived from left side only**

The main feature of the degenerative change in the vagal nuclei of the medulla oblongata was the failure of the neurones to take up the thionin stain. This can be seen readily even with low powers of the microscope. Pl. 1, fig. 1, shows almost complete degeneration of the cells of the dorsal motor nucleus on the left side in marked contrast to the normal cells in the right dorsal motor nucleus. This figure is of the medulla of sheep 3 where the left vagus was divided in the neck. Pl. 1, fig. 2, is from goat 21 where the ventral abdominal vagus was divided, and shows considerable numbers of degenerating cells in both right and left dorsal motor nuclei.

When examined with higher powers of the microscope the chromatolytic change is even more striking. The affected cells are swollen and being devoid of tigroid granules present a hyaline appearance. Often the nucleus is displaced to the side of the cell and it does not stain as darkly as the normal neuronal nucleus. In some degenerate cells vestiges of tigroid substance remain at the periphery of the cell immediately beneath the cell membrane (Pl. 2, fig. 4). Pl. 2, fig. 5, is taken from the nucleus ambiguus of sheep 3 and shows degenerating neurones similar to those of the dorsal motor nucleus. Many of the degenerate neurones can be seen only as 'ghosts' and it is even possible that some neurones have disappeared completely.

In the two sheep where vagal section was performed in the neck, retrograde degeneration occurred only in nuclei of the same side (Pl. 1, fig. 1). Following section of the right cervical vagus degeneration was almost complete in the dorsal motor nucleus of that side. On the left side, however, vagal section produced degeneration which was complete rostral to the obex, but caudal to this level only about half the cells were degenerate. The nucleus ambiguus is not normally easy to follow in the normal sheep medulla but the presence of chromatolytic cells following cervical nerve section made it more apparent. Only the nucleus ambiguus on the operated side was affected, the dorsal half of the rostral pole showing almost complete degeneration, but the extent of degeneration was considerably reduced posteriorly and at the caudal limit only very few cells were affected.

Where the dorsal abdominal vagus was divided, degeneration occurred in both right and left dorsal motor nuclei on the vagus. It was most marked in the rostral half of the nucleus, both right and left sides being affected to a similar extent. The few millimetres of the quarter of the nucleus immediately rostral to the obex showed the greatest amount of degeneration but here the cells situated dorso-laterally in the nucleus were unaffected. Posterior to the obex only a few cells showed degeneration, although most sections had occasional degenerate cells.

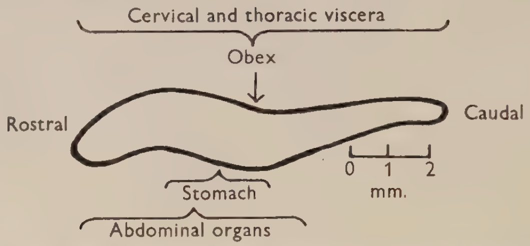
In goat 26 the dorsal abdominal vagus was derived from the left cervical vagal trunk only, the usual component from the right side was not demonstrable on post-mortem examination. Degenerative change was confined to the left dorsal motor nucleus. In front of the obex about 60 % of cells were degenerate and this degree of change extended to the anterior quarter of the nucleus but rostrally from this level the degree of degeneration fell away rapidly.

In goat 28 the left component of the dorsal abdominal vagus was divided, the right component being left intact. Chromatolysis was restricted to the rostral half of the left dorsal motor nucleus affecting less than 50 % of the cells of the middle quarter and below 25 % in the anterior quarter.

In the three cases where the ventral abdominal vagus was sectioned degeneration was somewhat variable, although in all cases both dorsal motor nuclei were affected. In goat, 21 the left nucleus showed up to 50 % of the cells in the rostral quarter of the nucleus to be degenerating, but in the next quarter it increased to 80 %, falling rapidly to zero immediately behind the obex. The right dorsal nucleus showed a similar pattern but with fewer degenerating cells. In goat 25 the degeneration was much less than in goat 21 and was again restricted to the anterior half of the nucleus. Goat 40 showed very few degenerating cells and these were seen immediately in front of the obex. In goat 21, but not goat 25 or goat 28, the nucleus ambiguus showed very occasional cells with retrograde change especially in the right side, in sections extending through the whole of the nucleus.

In goat 20 only the left component of the ventral vagus was divided. Very few degenerating cells could be found and these were located in both dorsal motor nuclei immediately rostral to the obex.

In goat 24 both dorsal and ventral components were divided. Degeneration occurred in both dorsal motor nuclei but was confined to those regions in front of the obex. About 50% of cells showed chromatolysis in the quarter of the nucleus immediately in front of the obex, but this fell to 30% in the rostral quarter. The nucleus ambiguus was unaffected.



Text-fig. 1. Diagram of the dorsal motor nucleus of the goat showing the approximate localization of motoneurones to the viscera of the thorax and abdomen.

DISCUSSION

From the retrograde degeneration which occurs in the vagal nuclei of the medulla oblongata following nerve section it is apparent that efferent fibres to the stomach and other abdominal viscera of the sheep take origin there. This is in agreement with the arrangement of efferent nerve fibres to the simple stomach of such animals as man, rabbit, cat and dog (Kuntz, 1953). In the sheep and goat the majority of motoneurones supplying vagal fibres to the abdominal viscera are situated in the oral half of the dorsal motor nucleus of the vagus, especially in that part of the nucleus immediately in front of the calamus scriptorius. The abdominal viscera, including the stomach, do not have an exclusive topographical representation immediately rostral to the obex for degenerating cells extend to the anterior limit of the nucleus. The thoracic and cervical viscera show a representation of cells throughout the length of the dorsal motor nucleus but especially in the posterior half. This becomes obvious from the distribution of degenerating cells following unilateral section of the vagal trunk in the neck (Text-fig. 1). This result agrees with that of Kitchell *et al.* (1956) who showed that in calves and sheep the cells supplying fibres to the abdominal organs are located principally in the rostral part of the nucleus and those supplying thoracic organs are located principally in the caudal part of the nucleus.

The vagal fibres from the nucleus ambiguus do not extend to the abdominal organs, but these nuclei do provide some motor fibres, probably to the larynx and oesophagus, in the cervical vagal trunk. The very occasional degenerating cell noticed in the nucleus ambiguus following section of branches of the abdominal vagus were probably not associated with the neurectomies but were the rare degenerate cells seen even in apparently normal nuclei.

The arrangement of the cells producing fibres to the stomach of the small ruminant reported here, is in agreement with the arrangement already described in the rabbit. Molhant (1910) observed that efferent axons to the stomach are derived from motor nerve cells in the anterior half of the dorsal motor nucleus. This was confirmed by Getz & Sirnes (1949) who found the abdominal organs represented throughout the length of the nucleus but mainly in that part of the nucleus immediately in front of the obex. In the cat, Mohiuddin (1953) states that 'no convincing evidence could be found that a greater proportion of cells become chromatolytic in one part of the nucleus than in another'. In the dog, Husten (1924) also concludes that the abdominal organs are represented throughout the whole nucleus. There appears to be no general topographical arrangement common to the species examined so far within the dorsal motor nucleus of those motoneurones supplying the abdominal viscera. The difference in the arrangements of motoneurones seem to be fairly wide and apparently depends upon variation between species.

In the goat and sheep the unilateral representation in the medullary vagal nuclei of axons occurring in the cervical vagal trunk is exactly similar to other animals. This finding agrees with the observation in the sheep that the motor effect elicited by central stimulation is mediated via the vagal nerves without any intramedullary decussation (Bell & Lawn, 1955). Furthermore, Bell & Lawn (1955) obtained most reticulo-ruminal responses from discrete loci in the dorsal lateral reticular formation extending rostrally from the obex for 2–3 mm. These authors were rarely able to evoke motor responses from the dorsal motor nucleus itself and concluded that the reactivity was due to stimulation of either afferent or efferent fibres in the reticular formation passing to or from the motor nucleus. The present study of retrograde degeneration of cells in the dorsal motor nucleus of the vagus does not resolve the ambiguity of the physiological results. It shows that motoneurones in the dorsal nucleus in the region that did not react to electrical stimuli do, in fact, provide efferent fibres to the reticulum and rumen. The motor responses mediated through these nerves, however, on stimulation of the reticular formation, could possibly result from the intramedullary stimulation of afferent fibres of the reticulo-ruminal reflex (Iggo, 1951).

The bilateral origin in the medulla oblongata of the axons in the dorsal and ventral abdominal vagi as revealed by these studies provides further support for the view of 'double innervation' of the stomach by the vagus (Mangold & Klein, 1927). Each dorsal motor nucleus supplies elements to both dorsal and ventral abdominal vagal trunks so that motor impulses are provided to the abdominal viscera if even one whole cervical vagal trunk is eliminated. The anatomical evidence derived from the retrograde degeneration studies reported here supports the finding of summation of motor response in the reticulum after stimulation of two medullary loci on either side of the midline (Bell & Lawn, 1955).

SUMMARY

1. Division of either the dorsal or ventral abdominal vagus does not prevent the regular contractions of the reticulo-ruminal sacs of the stomach nor is rumination interfered with.

2. Division of the vago-sympathetic trunk in the cervical region produces retrograde degeneration in almost all of the cells of the homolateral dorsal nucleus of the vagus. The contralateral dorsal nucleus is unaffected. A limited amount of degeneration occurs in the nucleus ambiguus of the same side but not on the opposite side.
3. Section of the dorsal or ventral abdominal vagus produces limited chromatolytic degeneration in both dorsal vagal nuclei. This bilateral origin in the medulla of motor fibres provides a double innervation of the ruminant stomach.
4. In the sheep and goat the majority of motoneurones supplying vagal fibres to the abdominal viscera are situated in the oral half of the dorsal motor nucleus of the vagus, especially in that part of the nucleus immediately in front of the calamus scriptorius. The thoracic and cervical viscera show a representation of cells throughout the length of the dorsal motor nucleus but especially in the posterior half.
5. The vagal fibres from the nucleus ambiguus do not extend to the abdominal organs but these nuclei do provide some motor fibres to structures in the neck.

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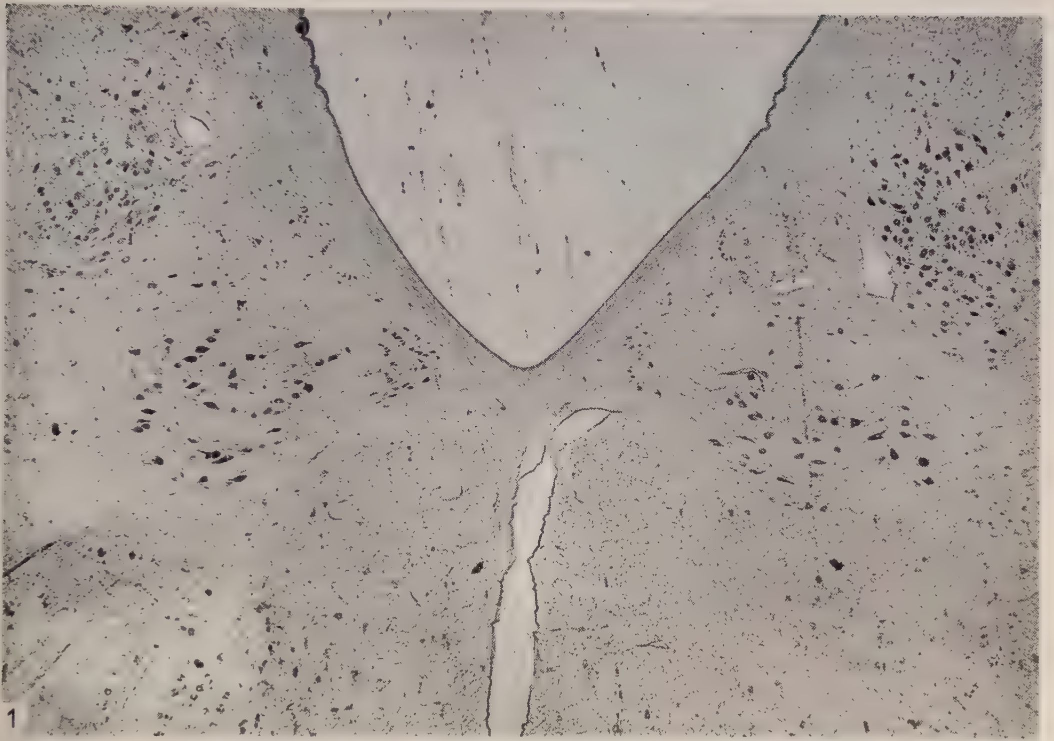
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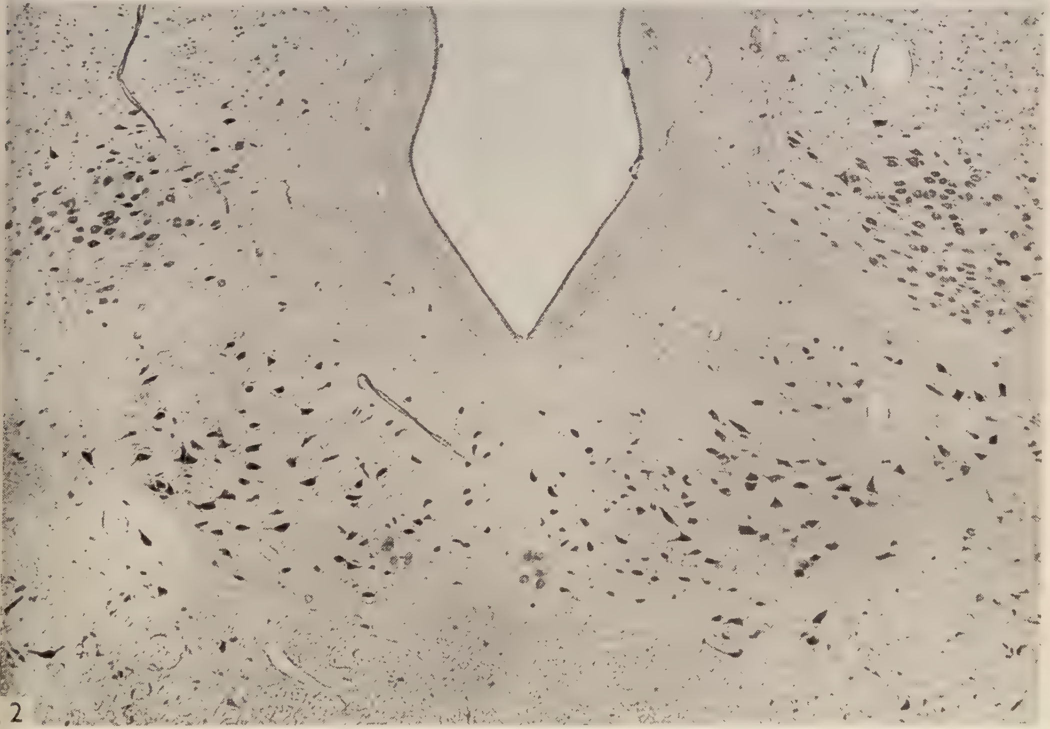
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BELL—LOCALIZATION WITHIN DORSAL MOTOR NUCLEUS

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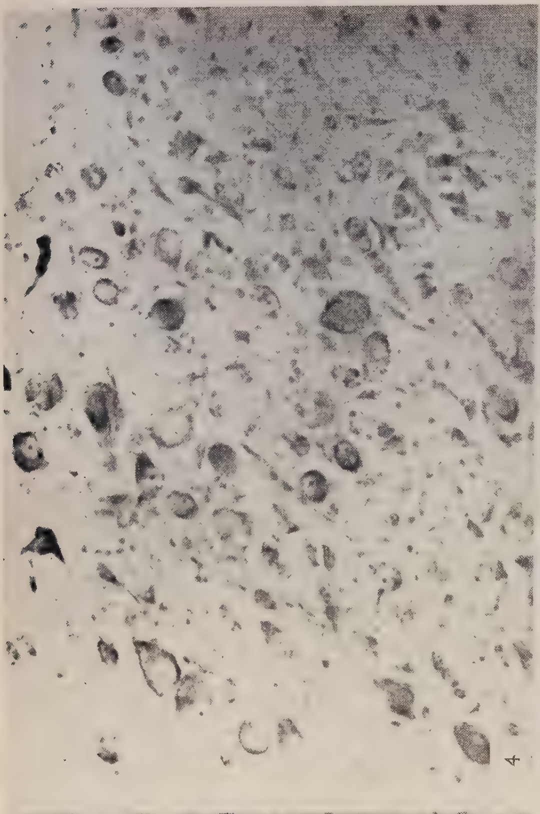

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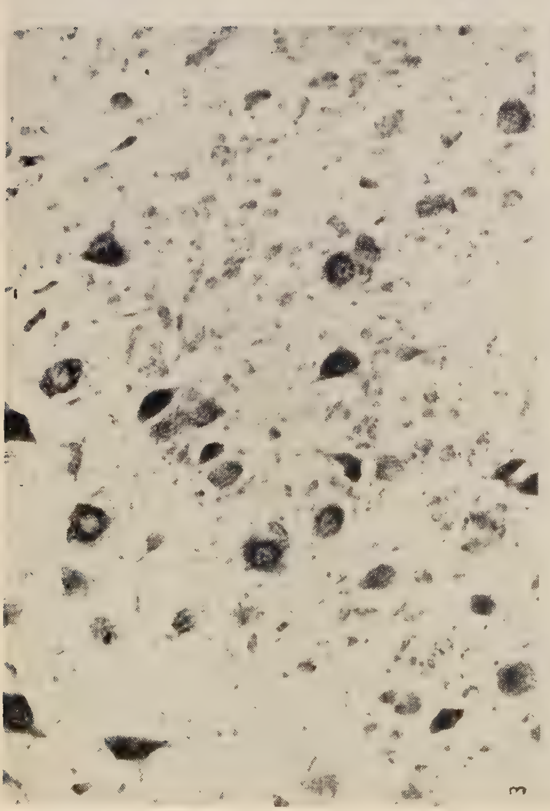

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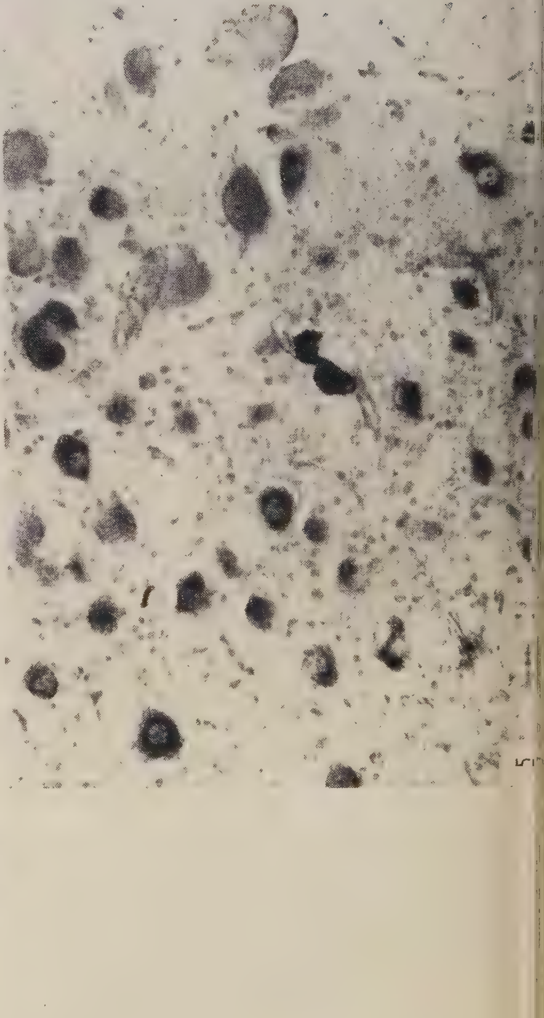

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BELL—LOCALIZATION WITHIN DORSAL MOTOR NUCLEUS

EXPLANATION OF PLATES

Plate 1

Fig. 1. Transverse section of medulla oblongata of sheep at caudal end of the 4th ventricle. The left dorsal motor nucleus of the vagus shows very marked degeneration following section of the cervical vagus on that side. The right dorsal motor nucleus is unaffected and stains normally. Thionin, × 25.

Fig. 2. Transverse section of medulla oblongata of goat slightly more caudal than that in fig. 1. Both right and left dorsal motor nuclei of the vagus show degenerating cells following section of the ventral abdominal vagus immediately rostral to the diaphragm. Thionin, × 25.

Plate 2

Fig. 3. The staining reaction of normal cells of the dorsal motor nucleus. Thionin, × 270.

Fig. 4. Chromatolytic cells of the dorsal motor nucleus following section of the cervical vagus on the same side. Thionin, × 270.

Fig. 5. Staining reaction of the nucleus ambiguus after section of the cervical vagus on the same side. Cells in the top right quadrant show chromatolytic change. Thionin, × 270.

OBSERVATIONS ON THE PLACENTAL GIANT CELLS OF THE RAT

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The trophoblastic giant cells of the rat placenta have been divided into two groups by Alden (1948). They differ in position and time of appearance, rather than in nature. The first set appears during the antimesometrial implantation and is derived from the ab-embryonic trophoblast. Alden called the cells of this set the primary giant cells, though it may be better to call them the implantation giant cells. They will later be related to the decidua capsularis. The cells of the other set, with which alone this paper deals, first become numerous at the 8th day (Alden), are derived from the ectoplacental cone and are applied to the mesometrial decidua over the whole area of the chorio-allantoic placenta. Alden termed these the placental giant cells. Though his study was mainly concerned with the implantation, or primary, giant cells, he ascribed phagocytic properties to the placental giant cells. These cells have also been described as phagocytic in the mouse (Snell, 1956) and in the hamster (Orsini, 1954). Bridgman (1948*a*, *b*) made a study of them in the rat. She found that they ingest trypan blue injected into the mother and that, at the 9th day, they appear to contain ingested red cells. Further, she states that they are involved at the 11th day in symplasma formation, ingesting or absorbing the cytoplasm of decidual cells and leaving masses of nuclei, which are later ingested.

In a study of the carbohydrate materials in the rat placenta, Bulmer & Dickson (1960) described PAS positive diastase fast granules, which gave a positive coupled tetrazonium reaction for protein, in the placental giant cells. These granules range in size up to approximately the diameter of an erythrocyte and they can so resemble erythrocytes in, for example, a trichrome stain that the conclusion is attractive that these granules represent the basis for suggestions of phagocytosis of red cells. Phagocytosis, either of red cells or of decidua basalis, by placental giant cells was not a striking feature of the material utilized in the study referred to. Evidence of it was sought, therefore, with the results described below.

The placental giant cells are of interest, not only because of their alleged phagocytosis of red cells and decidua but also because of their very gigantism, their possible polyploid condition and their apparently complete failure to multiply by mitotic division. Certainly Bridgman found no mitotic figure in a giant cell in her study of the rat placenta, even in colchicine-treated specimens. The finding of Orsini for the golden hamster was similar. Yet the layer of placental giant cells undergoes a manifold increase in area as the placenta grows. If the giant cells cannot increase in number by their own division, it would seem that the increase in area of the layer can come about by the sliding of the cells over one another, the layer increasing in area at the expense of its thickness, or by an addition to the number of giant cells from elsewhere during the period of growth of the placenta. In the former case, one might expect to find that not only does the giant cell layer become thinner as it increases in area but also that its individual cells become attenuated. In the investigation to be described, the thickness of the giant cell layer has been noted at various stages and measurements have been made in an attempt to discover whether the cells become altered in form. Since the form of the giant cell, while it is in general fusiform, is somewhat variable and since, moreover, the outline of the individual cells is not always distinct, the nuclei have been taken as indicators, as they were by Orsini in the hamster. It is probable that a minor attenuation of the cell would leave the nucleus unaltered and that, conversely, an attenuation of the nucleus would indicate a major alteration of the form of the cell.

MATERIAL AND METHODS

The material utilized was part of that used for the study of the carbohydrate material in the rat placenta (Bulmer & Dickson, 1960). Evidence of phagocytosis was sought in sets of trichrome-stained 5μ or 7μ sections extending through 10-, 12-, 14- and 17-day placentae, which had been fixed in 10 % formalin and embedded in paraffin. Cytoplasmic basiphilia was investigated by staining in toluidine blue, azur A or thionin. Ribonucleic acid was identified by digestion in a 0·1 % solution of crystalline ribonuclease (L. Light and Co.). With further sections, the ribonucleic acid was removed by a 15 min. hydrolysis in normal hydrochloric acid at 60°C.

The measurements of giant cell nuclei were made on trichrome-stained material. It was accepted that measurements of nuclei which may reach 60μ in length could not be of a high order of accuracy in 5μ sections. These measurements were regarded, therefore, as being in the nature of an attempt to indicate objectively something which was obvious subjectively, namely, that the giant cells and their nuclei increase in size during the period under study. It is likely that, in a 5μ section, only an occasional nucleus will show anything like its maximum dimensions. Accordingly, the thirty largest sections of giant cell nuclei were measured in a number of slides so spaced that no nucleus could be measured twice. The length and breadth of the nuclei were measured and their means taken as indicative of the size of the nuclei at the various stages.

OBSERVATIONS

At 10 days, a layer of giant cells, six or seven cells thick and containing maternal blood spaces, is applied to the decidua basalis. The interface between the giant cell layer and the decidua is smooth and featureless, there being no indications of invasiveness in the form of extension of giant cells, or even of cytoplasmic processes of giant cells, among the decidual cells.

There is no evidence, in the form of foreign nuclei or recognizable fragments of foreign cytoplasm, that the placental giant cells are ingesting decidua basalis cells. It is not possible to state categorically that no placental giant cell contains an ingested red cell, for every giant cell, it would appear, has at least one maternal blood space related to it and, if the interface lies obliquely to the plane of section, it may be exceedingly difficult or impossible to be certain on which side of the interface a red cell lies. Since evidence of phagocytosis to be acceptable should be unequivocal and since even equivocal evidence is very infrequent, it is best to conclude that, if it occurs, ingestion of decidua basalis cells or of maternal red cells by the placental giant cells is not of major importance.

The cytoplasm of the majority of giant cells contains numerous glycoprotein granules. The cytoplasm exhibits marked basiphilia, which is removed by ribonuclease. The nucleoli are extremely dense. Though there is considerable mitotic activity in the cells of the adjacent junctional (spongy) zone trophoblast, no mitotic figure is seen in a placental giant cell.

The mean dimensions of thirty of the largest sections of giant cell nuclei were: length 33μ, and breadth 15μ.

At 12 days the area of the interface between the giant cell layer and the decidua basalis is many times greater than at 10 days, but the layer remains six or seven cells thick. It is thus evident that the number of giant cells must be greatly increased. Not only have they increased in number, but they also appear to have increased in size, as indicated by the mean dimensions of the thirty largest nuclei observed: length 43μ, and breadth 18μ.

The cytoplasmic basiphilia due to ribonucleic acid appears to be as dense as at 10 days (Pl. 1, figs. 1, 2). The content of glycoprotein granules is, in general, still high, though there is now some individual variation in this respect, some giant cells containing very few or even no granules. It is interesting to note that these granules have now appeared in occasional junctional (spongy) zone trophoblast cells. These cells are striking in their pleomorphism. Some resemble giant cells in their size and form, but it is not necessarily these which contain cytoplasmic glycoprotein granules. Frequently the junctional zone cells which contain these granules appear to be otherwise indistinguishable from the majority of the other cells in the zone. They are scattered, apparently at random, through the thickness of the zone. All types of junctional zone cells show frequent mitoses. Mitoses in cells containing granules are not difficult to find, but have been noted only in the periphery of the junctional zone, immediately under the giant cell layer.

At 12 days, no unequivocal evidence was observed of phagocytosis, either of decidual cells or of maternal red cells.

The features of the placenta of 14 days distinguishing it from those of earlier stages are the very large vascular channels in the decidua basalis and the numerous polymorphonuclear leucocytes in these channels as well as in the maternal vessels of the giant cell layer. Since the latter are often obliterated by the shrinkage of fixation, the contained leucocytes frequently appear to a casual glance to constitute phagocytosed material contained in the cytoplasm of giant cells.

At 17 days, a radical alteration is noted in the placenta as a whole, for, while at 12 and 14 days there is a wide interval between the giant cell layer and the 'capsule' (Bulmer & Dickson, 1960) which is occupied by decidua, at 17 days the giant cell layer is applied to the capsule. The decidua basalis has effectively disappeared (Holmes & Davies, 1948).

The thickness of the giant cell layer now shows considerable variation from place to place around the periphery of the placenta, being four cells thick in a few places and only one cell thick or even absent in others. There does not appear to be any order or arrangement in this variability. The mean dimensions of the largest sections of nuclei at this stage are: length 63μ, and breadth 18μ. No evidence of phagocytic activity has been noted. The content of glycoprotein granules is greatly diminished, only occasional giant cells possessing any at all. It is noteworthy that a high content is present in some junctional zone trophoblast cells. There is now no evidence of mitotic activity in this zone. The ribonucleic acid content of the giant cells may be slightly reduced at this stage.

Many of the giant cell nuclei at this stage display an interesting effect (Schiebler, 1958), which varies in its degree from one nucleus to another. In its least developed form, it is evident as minute depressions of the surface of the nucleus. In more developed form, the depressions are deeper, giving the sectioned nucleus the appearance of containing a number of vacuoles (Pl. 1, fig. 3). These 'vacuoles' sometimes look like nucleoli in a trichrome preparation. However, the contents of the vacuoles show the finely fibrous structure of fixed cytoplasm, whereas the nucleoli are homogeneous. Moreover, in thionin preparations, for example, digestion with ribonuclease for just sufficient time to remove cytoplasmic basiphilia removes also the basiphilia of the contents of the 'vacuoles' but is insufficient to remove the basiphilia of the nucleoli.

These changes are displayed, usually, by giant cells which are applied to the capsule. Some giant cells are incorporated in the capsule and these display further degenerative changes. First, the density of the nucleolar material diminishes until it matches that of the cytoplasm, and, secondly, in some cases at least, the nucleus becomes fragmented, possibly by the growth and coalescence of cytoplasmic pockets (Pl. 1, fig. 4). The fragments later disappear. It would be interesting to know what controls this process, which is presumably degenerative, and whether it is the nucleus or the cytoplasm which is the active agent. On the face of it, the whole process looks like a cytoplasmic activity which has escaped from nuclear domination or which has been taken over by some other control.

DISCUSSION

The number of placental giant cells increases at least until the 12th day. Evidence is lacking that they increase by mitotic division. It would appear, therefore, that, like the original placental giant cells derived from the ectoplacental cone, they have heteromorphic precursors. The junctional zone trophoblast cells would seem likely to be these precursors, for they show evidence of active multiplication, the mitosing cells frequently contain the glycoprotein granules which the giant cells contain and they are themselves derived from the ectoplacental cone which produces the first generation of placental giant cells. It is of interest to note that the junctional zone cells can divide even when they contain a considerable amount of stored non-cytoplasmic protein, if it is legitimate so to refer to the glycoprotein granules. While it is well known that a cell with a heavy content of accessory material cannot divide, it has been shown that cultured fibroblasts can divide when the amount is not too great (Bensch *et al.* 1959).

Binucleate placental giant cells are occasionally seen (Bridgman, 1948*a*, *b*) and are not easy to account for if mitosis of giant cells does not occur, for the most economical hypothesis concerning their origin would be that they result from failure of cytokinesis in a giant cell mitosis. They could, however, result from failure of cytokinesis of a junctional zone mitosis, followed by growth of the resulting binucleate cell to giant cell size. In whatever manner they arise they could represent a mechanism by which giant cells become polyploid. This sort of mechanism was described by Beams & King (1942) in regenerating liver cells and by Fell & Hughes (1949) in cultures of infant mouse tissues.

During the period under study the layer of giant cells diminishes in thickness. At 10 days it is six or seven cells thick, while at 17 days it varies from nothing up to, in a few places, four cells thick. There appear to be two mechanisms involved in this decrease. In the first place, the production of giant cells comes to an end, as evidenced by the lack of mitotic figures in the junctional zone, and, in the second, giant cells are incorporated in the capsule. One cannot say whether the decrease is also partly due to the cells sliding relative to one another to maintain the integrity of the layer as it increases in area with placental growth. One can say that cellular attenuation is not involved to such an extent as to affect nuclear form, for the breadth of the nucleus appears to remain unaltered.

The length of the giant cell nucleus increases from about 33 μ at 10 days to about 65 μ at 17 days. The size of the whole cell is presumably increased proportionately, or more than proportionately, with the increase of the nucleus. This increase would entail the synthesis of a considerable amount of protein. The giant cells are also apparently producing protein-containing granules. It must be borne in mind that these granules may, on the other hand, represent the accumulation of material taken into the cells by pinocytosis. The giant cells, and also the cyto- and syncytiotrophoblast of the placenta, are ideally situated for exercising pinocytic properties, their plasma membranes being bathed in maternal blood. Until evidence of such activity is found, it would seem best to assume that the granules are actually synthetized by the giant cells. It may be, then, that the granular synthesis and the cytoplasmic increase with the growth of the cells are sufficient to account for their ribonucleic acid content. It would seem unnecessary to postulate, as did Wislocki, Deane & Dempsey (1946), that the cytoplasmic basiphilia of the giant cells, like that of the junctional zone trophoblast, is evidence of their synthetizing blood proteins for the embryo. The placental giant cells and the junctional zone trophoblast are remote from foetal vessels, which do not penetrate beyond the labyrinth. It is difficult to see how materials synthetized in either region could reach the embryo. One would have to postulate, as Bridgman did for glycogen, a handing-on of the material from cell to cell until an embryonic vessel was reached. Such a process would appear to be uneconomic in respect of energy requirements. It seems unnecessary, in any case, to postulate protein synthesis in the placenta on behalf of the embryo, for the multiplication and differentiation of its cells are evidence of its ability to synthetize protein for itself. Furthermore, Brambell & Hemmings (1954) showed that, in the rabbit, certain proteins—antibodies—reach the foetus by way of the yolk-sac placenta and not by the chorio-allantoic placenta. Cyto-chemical evidence of activity in placental cells is not necessarily evidence of a mechanism of transfer to the embryo. It might indicate no more than a metabolic activity of the cells or it might indicate the production of a substance, not for the embryo but, through an action on the maternal system, for the benefit of the embryo. One is tempted to take this view in the case of the placental giant cells, related as they are to maternal, and not to embryonic, blood. If their glycoprotein content represents a secretion, it would seem more likely that this secretion acts on the mother than on the embryo. It may be that the glycoprotein represents the substance having the luteotrophic activity demonstrated by Astwood & Greep (1938) and further investigated by Mayer & Canivenc (1950) and Ray, Averill, Lyons & Johnson (1955). This substance might, however, be expected to be PAS negative, for the representative of luteotrophic hormone in the rat pituitary gland is apparently PAS negative (Purves & Griesbach, 1951). The question of the nature of the glycoprotein remains open, then, and requires investigation by the parallel to Barrnett's work (1958) on the anterior pituitary, using cytochemistry and extraction procedures, with control by biological assay.

SUMMARY

The number of placental giant cells increases until the 12th day of gestation in the rat, apparently by production from the junctional (spongy) zone trophoblast. Production stops about the 12th day, after which their number diminishes. At least partially responsible for this is their incorporation in the 'capsule', during which their nuclei undergo a peculiar process of penetration by cytoplasmic ingrowths followed by fragmentation.

Satisfactory evidence of phagocytosis between 10 and 17 days is not forthcoming. It is suggested that one function of the placental giant cells may be the secretion of a glyco-protein hormone into the maternal circulation.

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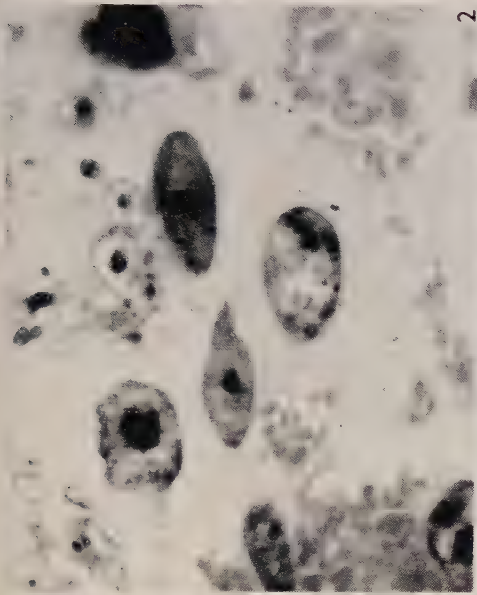
EXPLANATION OF PLATE

Fig. 1. Placental giant cells of a 12-day placenta. Toluidine blue, × 200.

Fig. 2. The same area as shown in fig. 1, in an adjacent section. Toluidine blue after ribonuclease digestion, × 200.

Fig. 3. Vacuoles in giant cell nuclei in a 17-day placenta. Trichrome, × 200.

Fig. 4. A fragmented nucleus of a giant cell undergoing incorporation in the capsule of a 17-day placenta. Trichrome, × 200.



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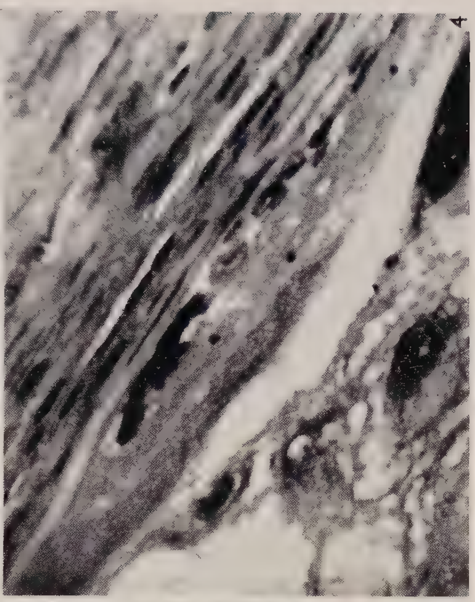

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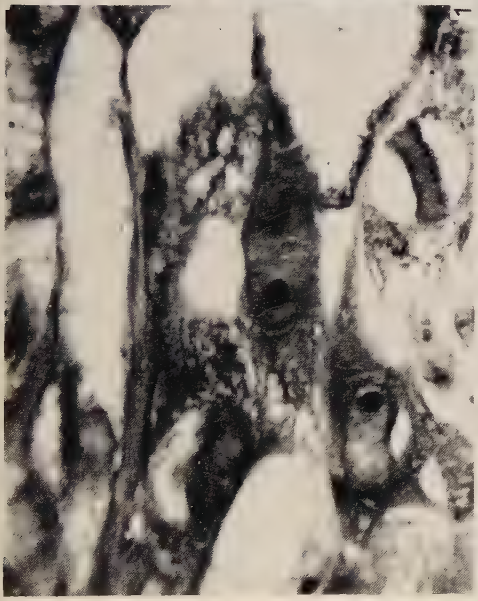

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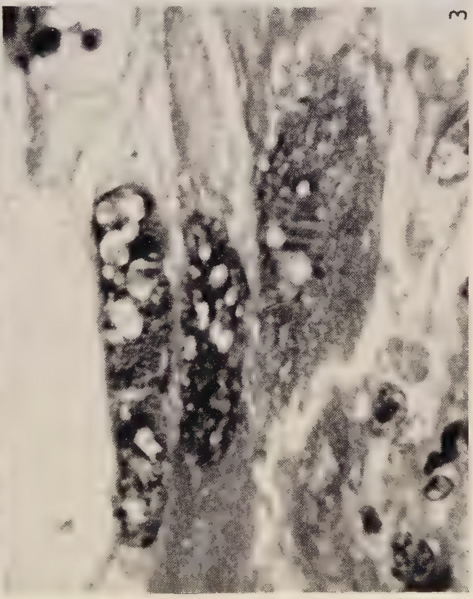

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DICKSON AND BULMER—OBSERVATIONS ON THE PLACENTAL GIANT CELLS OF THE RAT

(*Facing p.* 424)

ELECTRON MICROSCOPY OF THE UNUSUAL GOLGI APPARATUS WITHIN THE GASTRIC PARIETAL CELL

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INTRODUCTION

In 1954 two groups of electron microscopists (Dalton & Felix; Sjöstrand & Hanzon) identified in certain cells a characteristic cytoplasmic organelle at the site known to be occupied by the 'Golgi apparatus' of light microscopy. When first discovered, this organelle was identified as the 'Golgi apparatus', but subsequent evidence—reviewed later in this introduction—and the results of the present study, show that the two are not always identical, and therefore the organelle will be distinguished by the term 'Dalton Complex' (Baker, 1957*b*). The Dalton complex consists of (1) several flattened sacs which in section appear as parallel membrane pairs, (2) large vacuoles, and (3) small granules or vesicles, about 500 Å. in diameter. Since the original discovery, many workers have confirmed the existence of the Dalton complex in a wide variety of cells from both vertebrates and invertebrates (Hagenau & Bernhard, 1956; Rhodin & Dalhamn, 1956; Lacy & Challice, 1956; Lacy, 1957; Hally, 1958).

In a further electron microscopic study Dalton & Felix (1956), using a modified classical Golgi osmium method, found that post-osmication resulted in the deposition of reduced osmium tetroxide on the Dalton complex in several types of cell. Although the Golgi method consistently blackened the Dalton complex, the action on its several components was capricious. For example, in the exocrine cells of the pancreas the small vesicles were blackened, whereas in the epithelial cells of the epididymis it was the vacuoles and flattened sacs which were affected.

Lacy & Challice (1956) and Lacy (1957), applied unmodified classical silver and osmium methods to demonstrate the Golgi apparatus. They confirmed the findings of Dalton & Felix (1956) that the established Golgi methods blackened the Dalton complex, but once again, the reactions of the individual components were not uniform. In the exocrine cells of pancreas, silver methods blackened the flattened sacs, although as previously mentioned, Dalton & Felix had found that osmium methods blackened the small vesicles. Thus, in a single type of cell silver and osmium methods may give different results.

To sum up, electron microscopy has demonstrated in a wide variety of cells a morphologically distinct organelle—the Dalton complex. Classical osmium and silver methods used for demonstrating the Golgi apparatus consistently blacken the Dalton complex, although their detailed actions on the components of the complex are variable.

Light microscopists are generally agreed that a Golgi apparatus exists within the gastric parietal cell (Kolster, 1913; Beams & King, 1932; Menzies, 1949; Moussa & Khattab, 1957). It was therefore expected, in view of the foregoing evidence from other cells, that electron microscopy would reveal a Dalton complex. However, electron microscopical studies on the gastric parietal cells of the mouse (Challice, Bullivant & Scott, 1957; Kurosumi, Shibasaki, Uchida & Tanaka, 1958; Hally, 1959*a*, *b*) failed to demonstrate it, and thus posed the question: what organized structure—if any—represents the Golgi apparatus within the parietal cell? The present investigation, using a post-osmication method, is designed to resolve this problem.

METHODS

Healthy adult Swiss albino mice, aged 6–9 months, were fasted for 48 hr., and then fed. One hour after feeding they were killed by a blow on the head. Small portions of gastric mucosa, 1 mm.^{3} in size, were then placed in fixative within 2 min. of death. The specimens were fixed in isotonic buffered 1 % osmium tetroxide for 1 hr. After fixation, the specimens were washed briefly in distilled water, and divided into three groups. Each group was transferred to an aqueous 2 % osmium tetroxide solution, and incubated at 37° C. for 48, 15 and 3 hr. respectively. Following this post-osmication they were rinsed in distilled water, dehydrated through graded ethanols, and embedded in methacrylate in a routine manner (Hally, 1959*a*).

Thick sections were cut for phase-contrast and light microscopy.

Ultrathin sections were cut with a Cooke and Perkins ultramicrotome, and mounted on collodion-coated grids. A Philips EM. 75 B electron microscope was used at initial magnifications of up to × 12,000.

OBSERVATIONS

Light microscopy

After 48 hr. post-osmication, the parietal cells are easily distinguished from the other cells of the gastric tubule by the intense black granules of reduced osmium tetroxide—constituting the Golgi apparatus—which are scattered throughout their cytoplasm (Pl. 1, fig. 2).

After 15 hr. post-osmication, some of the parietal cells are heavily impregnated, but the majority show a few black granules in the cytoplasm which contrast with the grey mitochondria and clear canaliculi (Pl. 1, fig. 1). The other cells of the gastric tubule often contain a typical supranuclear Golgi apparatus (Pl. 1, fig. 1).

Thus, depending on the duration of post-osmication the Golgi apparatus of the parietal cell consists of (1) a few black granules, scattered throughout the cytoplasm, which contrast sharply with the mitochondria and intracellular canaliculi, or (2) numerous black granules practically filling the cytoplasm; in which case the mitochondria are no longer recognizable, although the intracellular canaliculi remain visible (Pl. 1; cf. figs. 1 and 2).

Electron microscopy

In moderately impregnated parietal cells, the Golgi apparatus consists only of black granules between the mitochondria (Pl. 2, fig. 4), but heavily impregnated cells contain—in addition to the black granules in the cytoplasm—blackened mitochondria (Pl. 1, fig. 3). Thus prolonged post-osmication leads to deposition of reduced osmium tetroxide within the mitochondria of the parietal cell.

Clearly, in moderately impregnated cells the 'Golgi apparatus' is represented by black granules in the cytoplasm. Let us, therefore, examine the relationship between these black granules and the other organelles of the parietal cell.

The cytoplasmic vacuoles of the agranular endoplasmic reticulum

A system of discrete vacuoles lies within the cytoplasm of gastric parietal cells which have been fixed by osmium tetroxide solutions (Sedar, 1955; Hally, 1959*a*). Although discrete vacuoles, they correspond to the agranular reticulum of other cells (Palade, 1956).

Following post-osmication these cytoplasmic vacuoles are no longer visible in moderately impregnated cells, but instead the black granules of the Golgi apparatus have a similar distribution within the cytoplasm (Pl. 2, fig. 4).

In order to ascertain the exact relationship between the black granules and these vacuoles, it is necessary to examine an earlier stage of post-osmication, before the Golgi apparatus becomes visible to the light microscope. After 3 hr. post-osmication small black particles only 50–200 Å. in size lie within the cytoplasmic vacuoles (Pl. 3, figs. 6, 7). Some particles appear to lie out with the vacuoles, but in a tangential section of a vacuole the obliquely cut membrane bounding the vacuole may not be resolved, and thus the contained particles will appear to lie free in the cytoplasm. Moreover, if free particles do exist one would expect to find them also in the cytoplasm near the periphery of the cell—where cytoplasmic vacuoles are sparse (Hally, 1959*b*), but in Pl. 1, fig. 3, there is a conspicuous absence of particles from this region. Thus the great majority, if not all, of these particles lie within the cytoplasmic vacuole.

Therefore the Golgi apparatus of the parietal cell is the agranular reticulum; rendered visible by the progressive deposition within its vacuoles of tiny particles of reduced osmium tetroxide.

Vacuole-containing bodies

These organelles contain black particles, even early in post-osmication (Pl. 3, fig. 6), and therefore make a small contribution to the image of the Golgi apparatus.

Lipid globules

Moderately blackened cytoplasmic inclusions, similar to those described as lipid globules by other authors, are observed within the parietal cell (Pl. 1, fig. 3). They are too small and sparse to contribute significantly to the light microscopic image of the Golgi apparatus.

Mitochondria

As already stated, there is a stage in post-osmication when the cytoplasmic vacuoles are blackened and the mitochondria are practically unaffected (Pl. 2, fig. 4), but as osmication proceeds the mitochondria blacken. The distribution of the black particles within the mitochondria is characteristic, for they are confined to the 'outer mitochondrial space', which is between the outer and inner mitochondrial membranes and extends into each crista. Particles are therefore seen within a crista, and never on the surface facing the mitochondrial ground substance (Pl. 3, fig. 5).

This selective blackening of the mitochondria only occurs within the parietal cell, for the mitochondria in the adjacent zymogenic cells are unblackened (Pl. 1, fig. 3).

DISCUSSION

The present study of the gastric parietal cell in the mouse shows that during post-osmication, reduced osmium tetroxide is selectively deposited in the agranular reticulum and the vacuole-containing bodies. As the latter occupy a very much smaller volume of the cytoplasm than the former, almost the entire Golgi apparatus is composed of the agranular endoplasmic reticulum.

However, with heavier impregnation the mitochondria are also blackened in a highly characteristic way. If one is not aware of this possibility, the blackened mitochondria could be erroneously included as part of the Golgi apparatus. This finding is not new: Bowen (1928) quotes numerous examples of cells in which the mitochondria or 'chondriomes' blackened if post-osmication or silver impregnation were prolonged. Some investigators have had difficulty in demonstrating the Golgi apparatus of the parietal cell (Beams & King, 1932; Menzies, 1949; Moussa & Khattab, 1957), and this may have been due to the tendency of the numerous mitochondria to blacken and thus obscure the 'true' Golgi apparatus—the agranular endoplasmic reticulum. Indeed, Moussa & Khattab (1957)—in a light microscopic study—describe a 'degenerate' Golgi apparatus in many parietal cells, and show micrographs where the cytoplasm is practically filled with blackened granules, many of which are probably mitochondria.

There has been much controversy about the detailed morphology of the Golgi apparatus in the parietal cell. Kolster (1913) describes a true reticulum dispersed throughout the cytoplasm: Beams & King (1932) found 'chiefly isolated filaments scattered fairly generally throughout the cytoplasm'; and more recently, Menzies (1949) found in the majority of parietal cells 'slightly osmophilic granules spread throughout cytoplasm and osmophilic strands between the granules'. This result of Menzies's is identical with the present findings: the 'slightly osmophilic granules' being the mitochondria, and the 'strands' the agranular endoplasmic reticulum.

The appearance of the Golgi apparatus—consisting as it does of the cytoplasmic vacuoles of the agranular endoplasmic reticulum—depends on the thickness of the section. The individual vacuoles are too close-packed to be resolved by the light microscope, and therefore in aggregates appear as strands or large granules between the mitochondria. Moreover, superimposition in 5 μ sections leads in some instances to an apparently complete reticulum throughout the cell.

The main finding of the present study, however, is that the Golgi apparatus of the gastric parietal cell is the agranular reticulum and not—as in other cells—the Dalton complex.

Although Baker and his colleagues (Baker, 1957*a*) have shown conclusively that histochemically the term 'Golgi material' is meaningless, in recent years the classical methods have been somewhat restored to favour by the discovery that they consistently blacken one or more components of the Dalton complex. The parietal cell is an exception, however, and thus one cannot assume that a cell contains a Dalton complex because classical Golgi methods have demonstrated that it possesses a Golgi apparatus.

Further, the fact that the Golgi methods blacken the Dalton complex in most cells and the agranular reticulum in the parietal cell, indicates that both may be related. The gastric parietal cell is not unique in possessing a diffuse vacuolar type of agranular endoplasmic reticulum, as cells in the adrenal cortex have a similar reticulum (Lever, 1955; Palade, 1956; Zelander, 1959; Carr, 1959).

Carr (1959) described within the 'compact' cells of the human adrenal cortex numerous vacuoles of the agranular reticulum lying between abundant mitochondria. He further observed, with the light microscope, that the classical Golgi apparatus consists of black granules which had the same distribution as the vacuoles.

Zelander found within the cells of the adrenal cortex of mouse numerous discrete cytoplasmic vacuoles in addition to groups of Dalton complex. He was unable to differentiate between the cytoplasmic vacuoles of the agranular reticulum and those belonging to the Dalton complex, as they were morphologically identical. Lever (1955) tentatively suggested that these cytoplasmic vacuoles within the cells of the adrenal cortex were the Golgi apparatus, but this hypothesis has never been tested.

Cytogenetic studies are also relevant, as Clermont (1956) concluded that within the developing rat spermatid, the Dalton complex gave rise directly to the cytoplasmic vacuoles of the agranular endoplasmic reticulum. Palade (1956), in a separate study of the rat spermatid, also produced evidence of the essential unity of the agranular reticulum and the Dalton complex.

Thus the above results and those of the present study provide evidence that the Dalton complex is a particular form of the agranular endoplasmic reticulum.

SUMMARY

1. The gastric parietal cells of the mouse were studied electron microscopically after using an osmium method to impregnate the Golgi apparatus.

2. Post-osmication results in blackening of the cytoplasmic vacuoles which are the agranular reticulum of the parietal cell. Thus the 'Golgi apparatus' of the gastric parietal cell described by light microscopists is the agranular reticulum.

3. This result is unexpected, as in recent years electron microscopists have demonstrated that classical Golgi methods consistently blacken a well-defined organelle—the 'Dalton complex'.

4. The significance of this observation is discussed.

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EXPLANATION OF PLATES

Micrographs of gastric parietal cells. Osmium tetroxide fixation, followed by post-osmication to impregnate the Golgi apparatus.

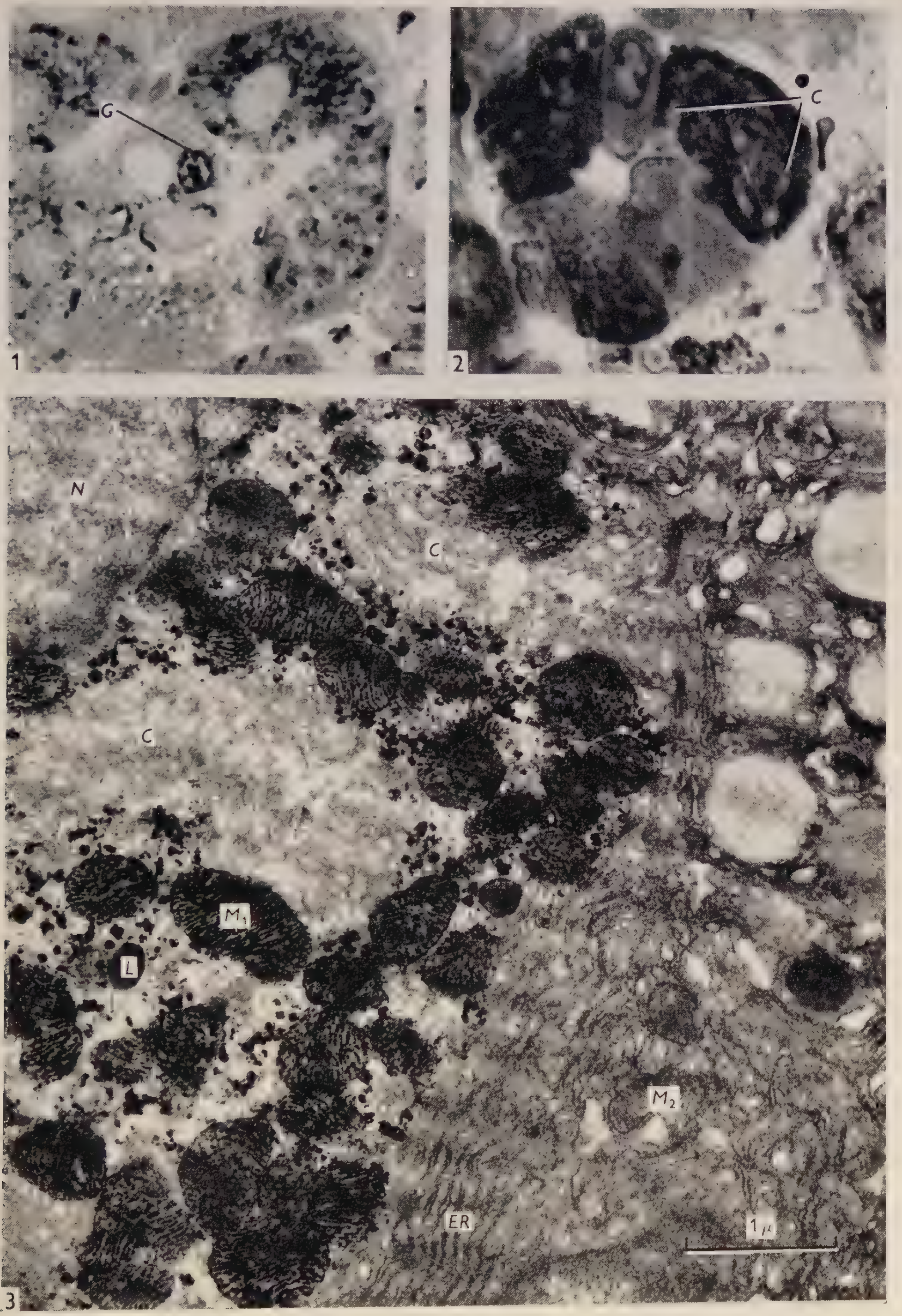
Abbreviations used

N, nucleus; *M*, mitochondrion; *C*, intracellular canaliculus; *V*, cytoplasmic vacuoles of agranular reticulum; *VCB*, vacuole-containing body; *ER*, granular endoplasmic reticulum; *G*, Golgi apparatus; *L*, lipid globule.

PLATE 1

Fig. 1. Light micrograph of gastric tubule showing two moderately impregnated parietal cells. Black granules and strands—constituting the Golgi apparatus—are scattered throughout the cytoplasm among the grey mitochondria and clear intracellular canaliculi. A cell adjacent to the upper parietal cell contains a typical localized Golgi apparatus (*G*).

Fig. 2. Light micrograph of gastric tubule with three heavily impregnated parietal cells. Their cytoplasm is filled with black granules which contrast sharply with the clear intracellular canaliculi (*C*).



HALLY—Electron microscopy of unusual golgi apparatus

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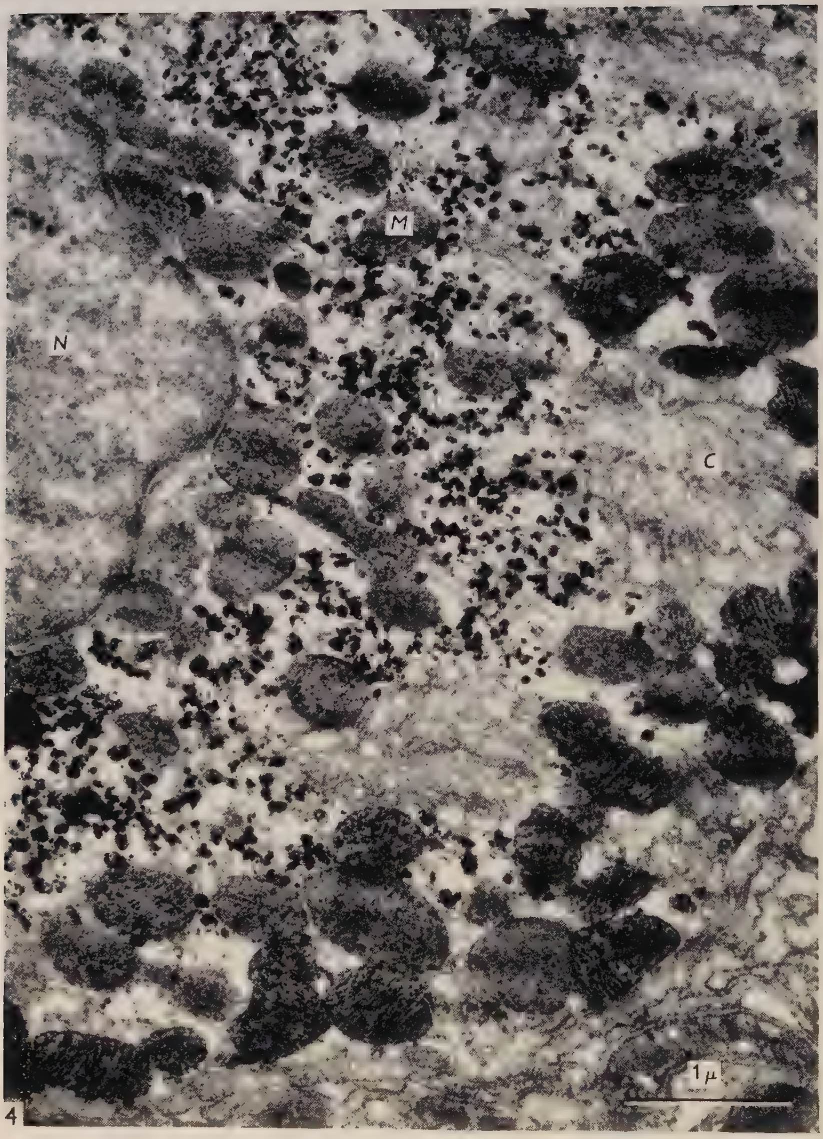

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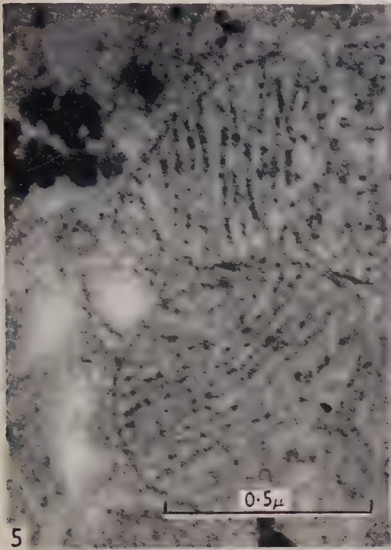
HALLY—Electron microscopy of unusual golgi apparatus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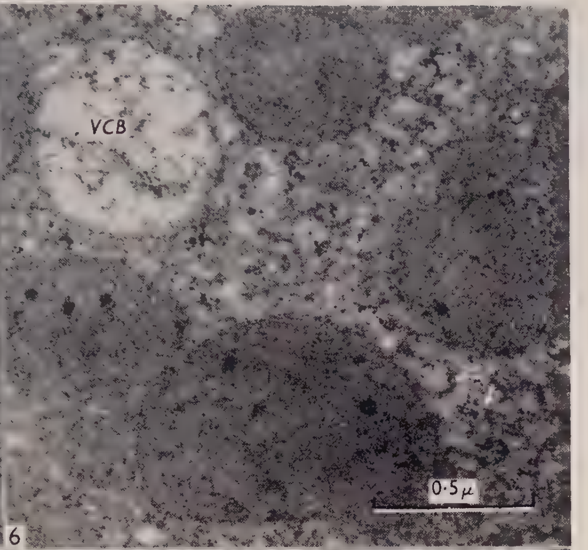
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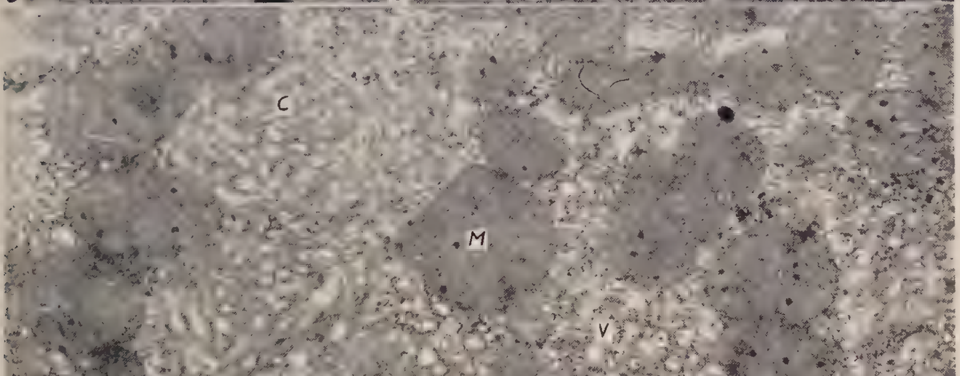
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HALLY—Electron microscopy of unusual Golgi apparatus

Fig. 3. Electron micrograph of heavily impregnated parietal cell comparable to fig. 2. Black granules lie between the blackened mitochondria (M_1). The intracellular canaliculi (*C*) and nucleus (*N*) are unblackened. A small lipid globule (*L*) is present. Observe that neither the granular endoplasmic reticulum (*ER*), nor the mitochondria (M_2), within the adjacent zymogenic cell are blackened. × 22,000.

PLATE 2

Fig. 4. Electron micrograph of moderately impregnated cell comparable to Fig. 1. Numerous black granules are found in the cytoplasm, but the mitochondria (*M*) are scarcely blackened. × 22,000.

PLATE 3

Fig. 5. This mitochondrion in a post-osmicated parietal cell contains characteristically situated black particles between the outer and inner mitochondrial membranes, and within each crista. × 54,000.

Fig. 6. Enlarged portion of fig. 7. Small metallic particles lie inside the cytoplasmic vacuoles of the agranular reticulum (arrows). A vacuole-containing body (*VCB*) also contains black particles, which are often arranged in delicate strands. × 43,000.

Fig. 7. Parietal cell after short post-osmication. Although numerous tiny particles lie within the vacuoles (*V*), they are rarely found in the mitochondria (*M*) or intracellular canaliculi (*C*). × 22,000.

ALIZARIN AS AN INDICATOR OF BONE GROWTH

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INTRODUCTION

Madder or its derivatives have long been used for the intra-vital staining of growing bone (Cameron, 1930). Alizarin, or its sodium salt, has more commonly been used during the past 100 years (Flourens, 1847; Gottlieb, 1914), and most workers have confirmed previous findings that only young or actively growing bone is stained red with the dye. Instead of giving alizarin orally, Gottlieb (1914) and Proell (1926), amongst others, gave sodium alizarin sulphonate—a water-soluble derivative—parenterally, and noted the rapidity of its action.

When given to a growing animal, alizarin stains the skeleton to an extent depending upon the dosage. Although young bones or parts of bones take up the dye better than the older parts, a single injection of a water-soluble compound in suitable amount can stain the whole bone system irrespective of age (Gottlieb, 1914). When a partly stained skeleton is studied in an animal sacrificed within a day or two of injection, the distinction between red (new) and white (old) bone is not always clear. It was this lack of precise definition between stained and unstained areas that led to the adoption of a technique in which the animal is allowed to survive for a period of days or weeks following administration of the dye: then the red bone (now 'old') is sharply contrasted with the new, white, bone. This technique followed naturally from the experiments of du Hamel (1739–43), of Hunter (1798), and of Macklin (1917), and was further elaborated by Brash (1924, 1926) and by Proell (1926).

Schour (1936), Schour & Hoffman (1938), Schour, Hoffman, Sarnat & Engel (1941), and most workers since, have used sodium sulphalizarate (1-2-dihydroxy-3-sodium sulphonic anthraquinone)—apparently identical with the sodium alizarin sulphonate used by Gottlieb in 1914. They gave it in a 2% solution in 0·45% sodium chloride by intraperitoneal injection, and reported that single doses of the order of 100 mg./kg. of body weight produced no significant toxic effects, though higher or repeated dosage did. Even single injections, however, gave a temporary retardation of gain in body weight in rats, with a subsequent return to a normal level. The dye was excreted in the urine and faeces, commencing within an hour.

Giblin & Alley (1942) did not find the madder method useful for measuring bone growth in the skulls of puppies. Madder feeding was too slow; intraperitoneal injections of alizarin too irritant (it is not clear whether they used insoluble alizarin or its soluble sodium salt) and purpurin gave poor results. They discarded the method. Nor did Robinson & Sarnat (1955) care for the alizarin technique. They said this about the use of madder (and, by inference, alizarin): 'Inclusion of madder creates an abnormal diet for the animals and may affect their growth pattern. The staining intensity varies with the age of the animal and the dosage of the dye used. *It cannot yield positive information regarding areas of absorption* [my italics]. Also, since resorption may lead to the removal of stained bone, vital staining will give incomplete data on the pattern of bone formation.'

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Jarabak & Vehe (1951) and Jarabak (1951) confirmed that sodium sulphalizarate can stain bone irrespective of age if used in suitable concentration, but found that the method did not compare in sensitivity with that employing radioactive calcium, (^{45}Ca), although this isotope was shown to be concentrated in the same areas as the dye. Dixon & Hoyte (1959) have shown that the information obtained from a study of the disposition of radioactive calcium is very similar to that obtained from alizarin-stained specimens, and that the two methods are complementary.

The present position is that the use of alizarin in correct dosage as an intravital stain for growing bone is well attested; that the bone stained by it contrasts well with that formed subsequent to its administration; and that its distribution is similar to that of radioactive calcium. However, the dye is toxic; it may affect normal growth and by the very nature of its deposition it gives information only regarding bone deposition and not bone resorption, except by inference (Brash, 1934).

It is usually stated that the whole skeleton of a young animal is uniformly stained red with alizarin or madder (du Hamel, 1740, 1741; Gottlieb, 1914; Brash, 1924; Massler & Schour, 1951; Mednick & Washburn, 1956), though Strelzoff (1873), Macklin (1917) and Proell (1926) had showed histologically that the dye was distributed unevenly. Histological studies were limited to examinations of bones or of portions of bones, cleared or uncleared, under the low-power binocular microscope, or to ground sections of bones (the dye is calcium-fixed, and is lost on decalcification). Serial sectioning of undecalcified bones stained by alizarin was described by Roberts & Hoyte (1958), and the study of these sections confirmed the uneven distribution of the dye.

Since the results of serial sectioning of undecalcified bones in this context have nowhere been published, the opportunity is now taken of showing in detail the distribution of the dye, especially in the bones of the skull, of evaluating its usefulness and of establishing histological criteria which signify bone deposition, stasis and resorption.

MATERIALS AND METHODS

Albino rats, rabbits and guinea-pigs were given a single intraperitoneal injection of a 2% solution of alizarin red (sodium sulphalizarate) at ages varying from birth to maturity, and were sacrificed at intervals following the injection. The dose was 50–100 mg./kg. of body weight (Schour, 1936). Gross specimens were prepared either by picking off the tissues after boiling in water, or by Green's antiformin technique (Green, 1934). Microscopic specimens were prepared from dried bones by using a high-speed carborundum cutting wheel (Hoyte, 1956, 1957), or from the whole heads of young animals by blocking in ester wax and sectioning with a special hard-steel knife on a sledge microtome. In this latter method (Roberts & Hoyte, 1958), 'Sellotape' was applied to the surface of the block prior to each cutting stroke. Serial sections varying in thickness as desired from 10 to 200 μ were obtained, each adherent to the 'Sellotape' (Pl. 1, fig. 1). The majority of the sections, cut at

$100\,\mu$, were mounted and examined without further staining. Others were stained, after removal of the 'Sellotape', with haematoxylin and eosin; with toluidine blue (with and without decalcification on the slide); with Van Gieson's stain or with Masson's trichrome stain; or by the von Kossa silver nitrate technique. Microradiographs of certain sections, still on the tape, were prepared, as described by Miller (1954) and Graham (1955). These sections could then be mounted and examined for comparison with the microradiographs.

Most of the sections sufficiently unfragmented for examination by these techniques were thick –50–$100\,\mu$—and therefore unsatisfactory for detailed histological examination of the soft tissues. For this reason, serial sections of the decalcified heads of control animals were prepared and stained in the usual way.

RESULTS

Evaluation of the use of alizarin in the 'labelling' of new bone

(1) *Rapidity of absorption and elimination*

Within 1 hr. of intraperitoneal injection of alizarin red into newborn animals, distinct coloration of the bones was observed. This colour appeared to reach its maximum in from 12 to 18 hr. In the fresh state it was reddish violet, but by the time the bones were prepared and dry, red was the predominant colour. The urine of the injected animals was seen to be coloured red within a few minutes, and was clear again within 24 hr.

(2) *Toxicity*

Variability of maternal care of the handled newborn animals made difficult an assessment of the immediate toxic effects of doses of the order of 50–100 mg/kg. However, weight records of the survivors showed that there was a temporary retardation of weight gain following injection, with a subsequent and speedy return to a normal rate of increase.

There is no doubt, however, that microscopic evidence of retardation of bone deposition can be found to confirm the toxic nature of the drug, even at these relatively 'safe' levels. In some rabbits injected on the first day of life, there was very little new bone formation even by the 5th day after injection, as seen by the virtual absence of new, white bone deposited upon the red. This is surprising, in view of the normal rapid increase in brain weight and of skull size in the neonatal period, and is to be correlated with the temporary retardation in weight gain. That it does not interfere with the usefulness of the dye as an experimental tool will be seen from the description given below.

(3) *Deposition of alizarin red in bone*

The dye is deposited in a single layer of bone, and indeed it is frequently apparent that it occurs in a single lamella. Even when given to newborn animals, in whom the entire skeleton appeared red, the microscopic picture revealed that there was still a deposit of red bone upon the previously existing, unstained, white bone (Gottlieb, 1914, quoting Strelzoff's observations; Proell, 1926; Hoyte, 1956). In certain areas of rapid growth, in the skull or at the epiphyses of long bones, whole trabeculae were coloured red. These bony bars were rarely more than one or two lamellae thick, and were probably formed at one time throughout their thickness.

Diffuseness of staining may be due to several causes. It may follow from the slow and continued absorption of the dye after its administration—either from the use of madder root by mouth or from the formation of a tissue depot deliberately (in subcutaneous or intramuscular injections) or inadvertently (as in some of the present series of experiments). Thus, in some of the newborn animals, there was a gross escape of the dye at the time of injection, into the subcutaneous or extraperitoneal tissues, seen when these animals were sacrificed in the early hours following its administration. Sections of the skulls of some animals, injected at birth and killed towards the end of the first week, showed, apart from the obvious red surfaces, diffusely pink trabeculae. The disposition of these showed them to be new, and it would seem that the alizarin was circulating for a longer time after the initial injection than one had supposed, and that in these too a tissue depot had been formed. (It seems unlikely that dye could be released by resorption from the skeleton in sufficient amount to effect appreciable staining of new bone in this way.)

Diffuseness of staining may, of course, be a false appearance, when seen by the naked eye or at low magnification, due either to the close aggregation of stained Haversian systems or of red trabeculae, to the red bone being seen through a thin layer of white, or to oblique sectioning. It may be a false appearance, due to the bone being cleared (or merely wet) prior to examination, when the clear distinction between red and white bone is lost. Paradoxically, clearing reduces the distinction between red and white bone in the 'red' areas, but increases the sharpness of contrast between these areas and adjacent, wholly white ones.

The dye is deposited on surfaces—subperiosteal, endosteal, on trabeculae flanking marrow spaces, around Haversian canals—known to be associated with the laying down of new bone. In sections counterstained with other dyes on the slide, the presence of osteoblasts in immediate contact with the newly laid down red trabeculae is evident (Pl. 2, fig. 9). In most of these areas, in specimens taken from animals surviving for longer periods, the red lines have been submerged by lamellae of white bone, where they have not been resorbed in whole or in part.

(4) *Microradiography of sections*

Microradiographs show that the red bone deposited on certain surfaces, especially that deep to the periosteum, is relatively translucent to X-rays (Pl. 1, figs. 2, 3). In the interpretation of these features in the microradiographs much care was taken to find areas in which there were no overlapping bone shadows to render the contrast between radio-lucent and radio-opaque bone too difficult. With this reservation, it is probable that the radio-lucent bone is recently formed and poorly calcified (Amprino & Engström, 1952; Amprino, 1953; Vincent, 1955). In animals surviving for longer periods, microradiographs of sections show that the red lines in certain areas have become more radio-opaque. They correspond now in general to areas of more highly calcified—older—bone, and are submerged in turn by more recently formed, radio-lucent bone. (It must be emphasized that there is no special feature of bone containing alizarin that distinguishes it on X-ray. It was thought that the injection might leave a 'mark' in the bone analogous to 'Harris's lines' (Harris, 1933), and support for this idea was suggested by the observations of Paff & Ecksterowicz (1950) and of Paff, Angulo & Ecksterowicz (1951) that alizarin prevents osteogenesis in tissue culture. However, no such 'mark' was found. One can only correlate the red lines on the actual sections with the trabeculae visible on the microradiographs of the same sections.)

(5) *Duration of staining with alizarin red*

When injected suckling or weanling animals were allowed to reach maturity before being sacrificed, it was seen that the red stain persisted in certain areas. The dye does not appear to be removed except in the course of normal resorption of bone.

(6) *Specificity of alizarin red for growing bone*

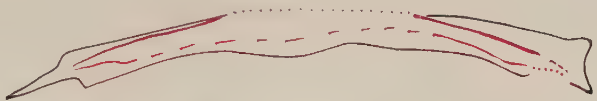
In the doses used, the dye did not appear to stain any bone other than new or growing bone. When injected into adult rats, it was still taken up only by bone developing at the epiphysial plate (in the albino rat the epiphyses do not fuse—Strong, 1925); at periosteal, endosteal and Haversian building sites (Hoyte, 1956).

Histological findings in animals injected with alizarin red

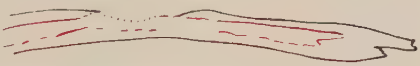
On examining the surfaces of the bones in the sections of the various skulls, different appearances were noted, depending on how long the animals had been allowed to survive between injection and sacrifice.

In skulls sectioned in the first few days following injection, the red-stained bone is that actively growing at the time of injection and circulation of the dye. Some areas presented red trabeculae deep to the periosteum, at varying angles to the surface. The appearance is one of rapidly spreading growth, and has been termed 'spreading trabecular growth' (Pl. 1, figs. 4, 5). In other areas the bone deposited deep to the periosteum consisted of a flat layer, varying somewhat in thickness in different situations. This is called 'flat subperiosteal growth' (Pl. 1, fig. 4). The presence of a completely stained surface implies that that surface was growing at the time of injection. Certain surfaces, or parts of surfaces, showed no stain at all. These were either static or resorbing areas, and in many areas of these young specimens no certain differentiation could be made between these two processes. The absence of stain does not necessarily mean, of course, that it has been removed—it may never have been deposited there. Sometimes, however, it is clear that one or other process is present. Stasis may be revealed by the absolute flatness of the unstained surface, resorption by the presence of scalloping (Howship's lacunae) (Pl. 2, figs. 7, 8). A resorbing surface is not usually completely unstained—there are usually red trabeculae or Haversian systems present. In general, the term employed in these early specimens is 'stasis/resorption'.

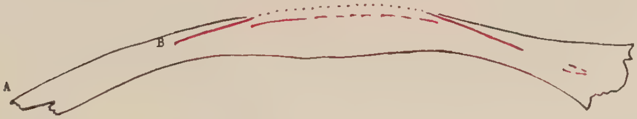
In sections from older animals, in which more time has elapsed between injection and death, both spreading trabecular and flat subperiosteal growth (of white bone upon red) can be seen. The difference between stasis and resorption is often more easily discerned. In the former, the static area may persist as an unchanged red surface, usually linear. In the latter, resorption is shown by the removal of red bone from areas where it previously existed in comparable sections; by the presence of red lines 'disappearing' at a surface or at an edge (Text-fig. 1); or by an area which shows marked scalloping, with portions of red trabeculae or stained Haversian systems left (such an area may still appear wholly red to the naked eye). Sometimes an area of previous stasis or resorption is overlaid by new bone—whereupon a typical reversal line is seen at the junction of the two. No attempt was made to assess further resorption of any of the new, unstained bone laid down: it is preferable to get a continuity of information by varying the ages of injection and sacrifice, so as to assess always the white bone in relation to the red.



Text-fig. 1. Longitudinal section through intermediate third of parietal of a rabbit, injected at 18 days; survival 25 days; anterior edge to right. The inner outline represents the alizarin-stained bone, the outer new growth; . . ., represents surface resorption; – – –, the interruption of the alizarin lines by the diploe. × 27.



Text-fig. 2. Longitudinal section through parietal of a guinea-pig, injected at 8 days; survival 42 days; detail of coronal edge. Symbols are as in Text-fig. 1. × 36.



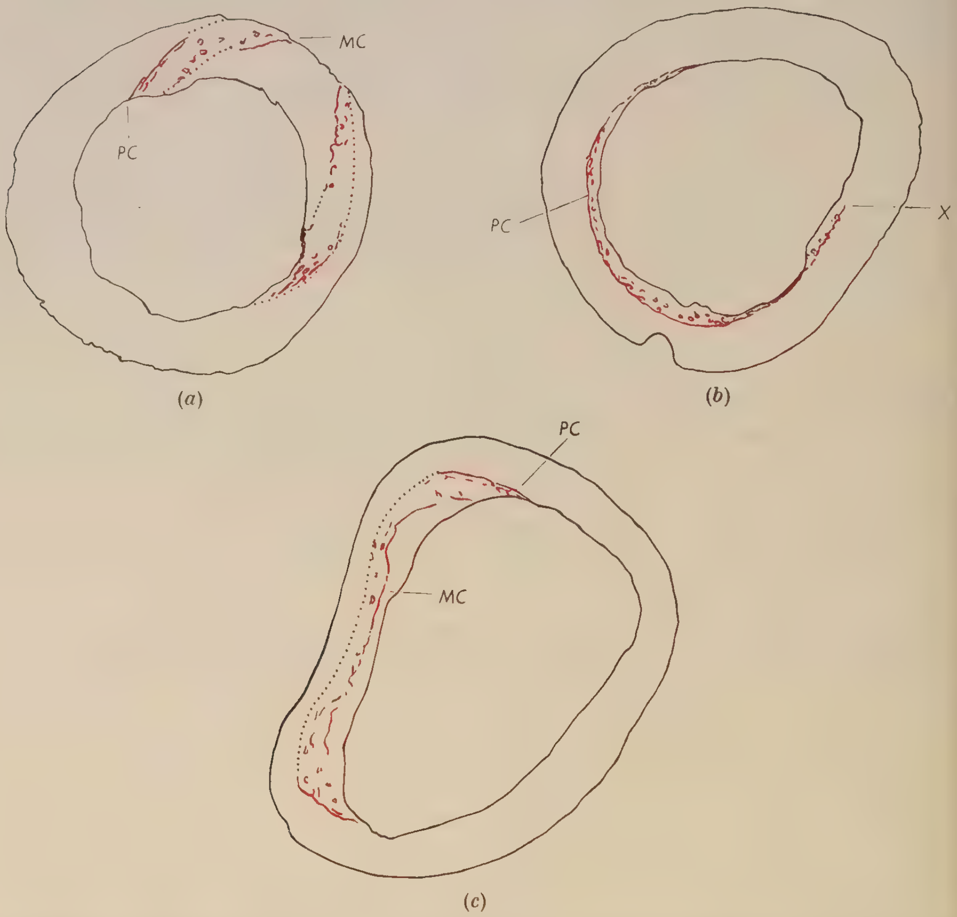
Text-fig. 3. Longitudinal section of parietal, through area of maximum convexity, in a rabbit injected at 1 day; survival 49 days; anterior edge to left. *A–B* is the apparent sutural increment. × 27.

In examining sections of the separate, dried bones from older animals, prepared on the carborundum cutting wheel, these and other criteria were recognized. In the bones of the skull vault red lines were seen marking the original inner and outer tables. Red trabeculae or occasional Haversian systems 'label' the area between the tables, and its subsequent development or removal could be traced. The inner and outer tables were sometimes joined together at or near the sutural edge by a 'closing' trabecula (Text-fig. 2). When seen, this can be taken as a fixed point. In its absence (Text-fig. 3), no conclusion can be reached as to the previous edge of the bone; no macroscopic view of the remaining red edge can be taken as a fixed margin; no measurements taken from such a 'margin' can be regarded as reliable.

In the long bones of these animals (Text-fig. 4) the fate of the red 'metaphysial cone' (Leblond, Wilkinson, Belanger & Robichon, 1950) and of the subperiosteal alizarin deposit was followed in serial transverse sections. Even if both lines have been resorbed, the bone which lay between them is clearly 'labelled' (as in the skull), and moreover is separated from a further layer of new bone deposited upon it by a distinct reversal line.

Histological findings in control animals

These criteria of surface changes in sections of alizarin-stained skulls and other bones were amply confirmed by the histological examination of control specimens. Spreading trabecular growth (Pl. 2, fig. 10) and flat subperiosteal growth (Pl. 2, fig. 11) are easily seen, and stasis or resorption can be recognized. Stasis is shown by the absence—absolute or relative—of osteoblasts at a bone surface, or by the persistence of woven bone at one surface whilst subperiosteal lamellar bone is being laid down at the opposite aspect (Pl. 2, fig. 11). Woven bone was recognized in sections stained with haematoxylin and eosin by the uneven staining of its matrix and its patchy basiphilia (Ham, 1957), or, in addition, when of endochondral



Text-fig. 4. Transverse sections of the left humerus of a rabbit, injected at 25 days; survival 73 days. (*a*) is through the upper third, (*b*) through the middle and (*c*) through the lower third of the shaft. ..., represents a reversal line (deposition upon previous resorption); *PC*, the original alizarin-stained periosteal surface; and *MC*, the similarly stained endosteal aspect (metaphysial cone); at *X* the alizarin line disappears in the thickness of the bone. × 27.

origin, by the cores of calcified cartilage within many of the trabeculae. Reversal lines are frequently seen in specimens (Weinmann & Sicher, 1955).

Though recognizing that the role of the osteoclast in resorption is not finally settled, the whole experience here summed up of the comparison of alizarin-stained specimens with normal controls leads one to agree with Le Gros Clark (1958) that the presence of osteoclasts 'in areas of localized bone absorption...is so consistent, and the histological picture they present is so persuasive, that there can be little doubt of their participation in the removal of bone'. The presence of osteoclasts was deemed evidence of resorption, and they were found in areas interpreted as resorbing in the alizarin-stained specimens (Pl. 2, figs. 7–10; Pl. 1, fig. 6). Sometimes stasis and resorption existed side by side. Sometimes resorption and growth existed at a surface in very close proximity to one another (Pl. 2, fig. 12) (see also Kirby-Smith, 1933).

DISCUSSION

The use of alizarin as a research tool in the study of bone growth has by no means been superseded, even in these days of the ready availability of radioactive isotopes. The ease and safety of its administration need no emphasis, and, in suitable concentration, it is not too toxic to be followed by normal growth. Like so many other techniques, it requires histological study, both in sections containing the dye and in sections from uninjected control animals. The preparation of sections of undecalcified bones is a problem common to all methods which employ substances bound to the inorganic constituents of bone, but it is not insuperable, as the above results, based upon a study of serial sections of bones so prepared, abundantly show.

Although many of the positive findings arising out of the study of gross specimens stained in life with this dye, or by madder root, have been known for 200 years, there was a need for a detailed assessment of their reliablity, based as they were upon a macroscopic distribution of the dye. Where exactly was the dye deposited, and how persistent was it? Were the lines of its deposition sufficiently clearly marked and permanent to be used for the measurement of growth? Were there indeed any fixed points in the growth of any bone?

On the negative side—that is, when considering bone 'subtraction' rather than bone 'addition'—could this method of intra-vital staining give reliable information about the sites and extent of bone resorption? That this *is* so follows from a consideration of the results listed previously, and will be further elaborated in later communications. It has been insufficiently recognized that the mere presence of red coloration in a bone might signify deposition of bone, stasis or even resorption, and therefore many workers (amongst them especially Massler & Schour (1951) and Mednick & Washburn (1956), who repeated some of Brash's (1924, 1934) work on the braincase of the infant pig) totally misconstrued surface changes. No measurements which rely upon the distribution of alizarin in bones can be considered sound unless microscopic evidence of that distribution in similar bones can be directly compared.

The great drawback to the use of alizarin in studies of bone growth is the difficulty of recording, and hence of publishing, the results: only in colour can the full value of such studies be realized. However, given a reliable qualitative assessment of the location of the dye, and of changes in bone surfaces in relation to its sites of deposition, it should be possible in a series of similar bones to show by measurement the amounts of new bone added and the directions of growth.

It can be concluded that alizarin red, given by intraperitoneal injection to growing animals, in doses of the order of 50–100 mg./kg. of body weight, on a single occasion, has a distinct sphere of usefulness:

(i) It is rapidly absorbed, rapidly fixed in the growing bone and the excess rapidly excreted.

(ii) It is not so toxic as to prevent subsequent normal growth in relation to its sites of deposition.

(iii) Its staining effects are precise, and limited to a single layer of bone being laid down at the time of its circulation in effective concentration.

(iv) The lamellae in which it is deposited show no abnormality to X-rays; nor does its presence prevent the continued normal calcification of these lamellae during growth.

(v) It persists in the lamellae of bone which it stains until removed by the normal processes of resorption.

(vi) By its use areas of resorption can be readily recognized, and, provided histological sections can be prepared, precisely delimited.

(vii) With it, areas of stasis can be shown, and by the judicious selection of a graded series of injected animals, 'fixed' points can be ascertained. Thus the patterns of bone growth may be followed, and measurements may be made with some degree of reliability.

SUMMARY

Alizarin red was given by intraperitoneal injection to growing animals, in doses of the order of 50–100 mg./kg. of body weight. Its distribution was studied in serial sections of the undecalcified skulls and limb bones. It is shown to be an intra-vital stain of some precision, rapidly absorbed, fixed only in the bone forming during the time of its circulation in effective concentration, and removed only in the course of normal resorption. Growth of the animals after injection, temporarily retarded, quickly became normal; and subsequent histological and microradiographic appearances were normal also.

The different appearances of the red-stained surfaces are noted, and criteria suggested that enable deposition, stasis and resorption to be recognized. These appearances were confirmed by the examination of sections from decalcified controls. Alizarin, used in this way, therefore, can give precise information about patterns of bone growth and resorption, and may permit of exact measurements of these activities.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Cutting undecalcified sections from an ester wax block. A 'Sellotape' ribbon is seen.

Fig. 2*a*. Microradiograph: transverse section of interfrontal suture in a 2-day-old rabbit injected with alizarin at birth. A core of radio-opaque bone runs through the frontal. ×36.

Fig. 2*b*. Drawn from the same section. All surfaces are red, the centre white. The spreading trabeculae are red, new and relatively radiolucent. The dense core consists of older, unstained bone. (The unbroken line —— represents flat subperiosteal growth; | | | or × × × spreading trabecular growth; and ··· stasis or resorption.) ×27.

Fig. 3*a*. Microradiograph: transverse section of frontal bone from the same rabbit, near front of orbit. Note the sharp, 'punched out' appearance of a resorbing area, and the radiolucent border superiorly (flat subperiosteal growth). ×36.

Fig. 3*b*. A drawing of the same section (symbols as above). ×36.

Fig. 4. Same animal as in fig. 2. Transverse section of mandible, inferior border. There are red trabeculae spreading medially (to left of photograph), and a flat, red subperiosteal layer laterally (right). ×36.

Fig. 5. Same animal as in fig. 2. An area of spreading trabecular growth, entirely red, cut transversely at the anterior extremity of the maxilla. Note the 'fading' of the red trabeculae into the tissues. ×36.

Fig. 6. One-day-old control rabbit. Detail of area shown in Pl. 2, fig. 10. Osteoclasts are seen. Haematoxylin and eosin. ×216.

Plate 2.

Fig. 7. Same animal as in fig. 2. Transverse section of mandible, showing spreading trabecular growth on the lingual aspect (right), scalloping on the crypt surface. ×36.

Fig. 8. Detail of lingual wall of crypt. ×90.

Fig. 9. As in fig. 8; counterstained with Toluidine blue. Note the intense periosteal reaction on the lingual surface and the presence of osteoclasts (arrowed) laterally. ×90.

Fig. 10. One-day-old control rabbit showing an area similar to the preceding. Note osteoclasts in the tooth crypt. Haematoxylin and eosin. ×90.

Fig. 11. Six-day-old control rat. Horizontal section of the parietal bone, just behind the coronal suture. There is flat subperiosteal growth externally (left), with numerous osteoblasts; and relative stasis endocranially (right), with few osteoblasts and persistent woven bone. Haematoxylin and eosin. ×216.

Fig. 12. One-day-old control rabbit. Transverse section of frontal bone, in medial wall of orbit. The osteoclasts lie on the orbital (left), the osteoblasts on the endocranial aspect of the thin plate. Masson's trichrome. ×216.

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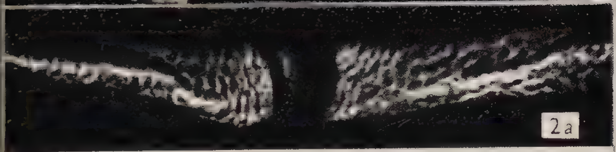
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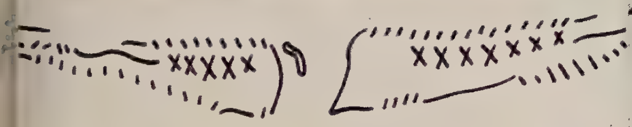
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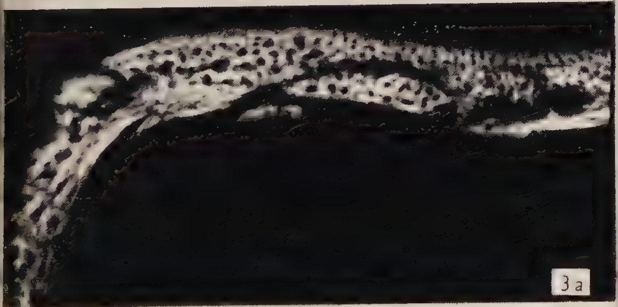
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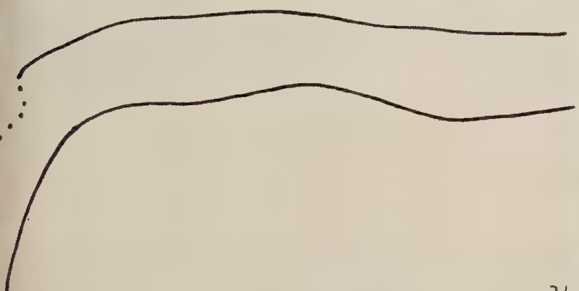
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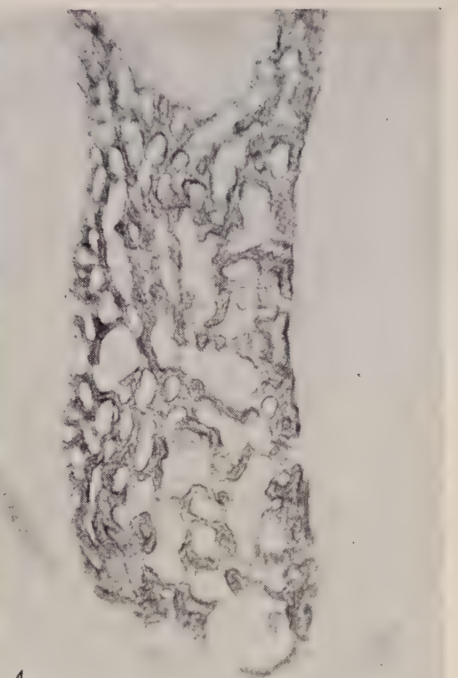
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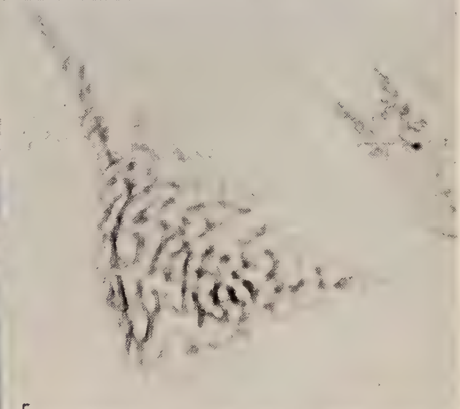
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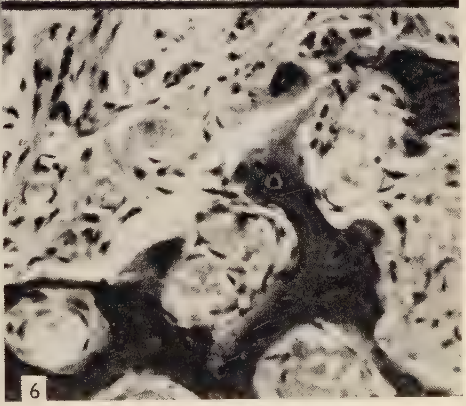
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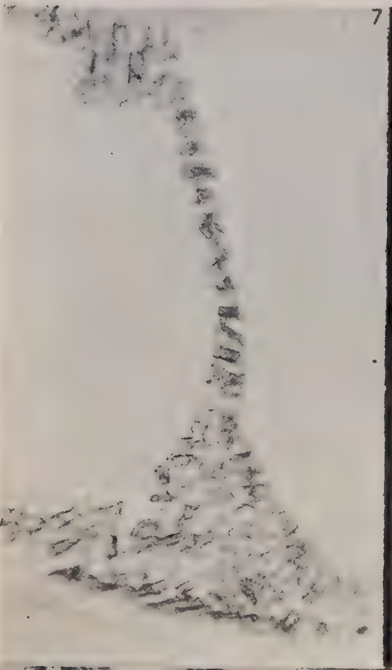
HOYTE—Alizarin as an indicator of bone growth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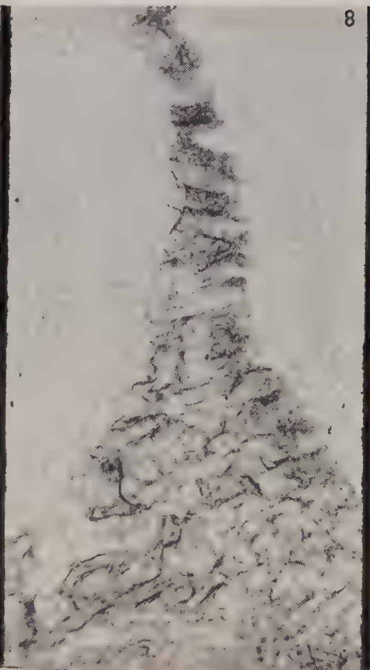
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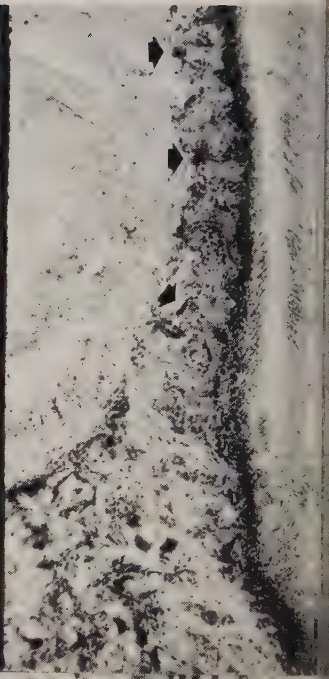

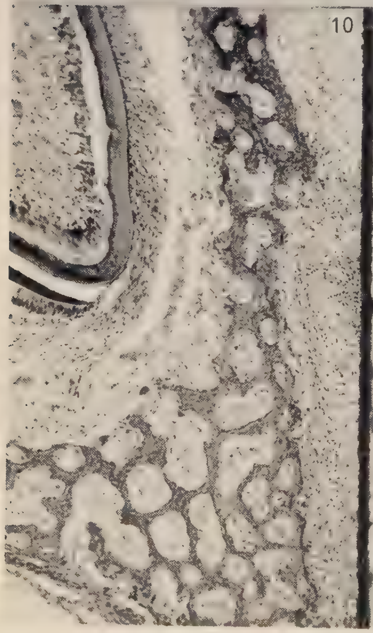
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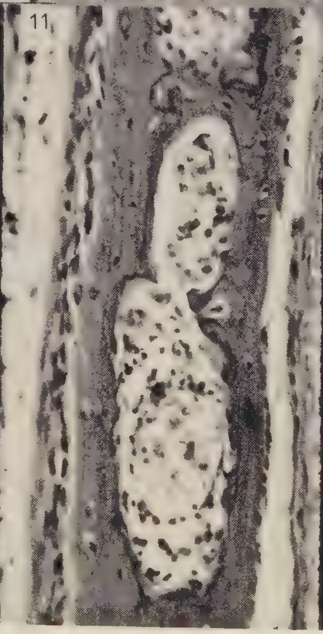
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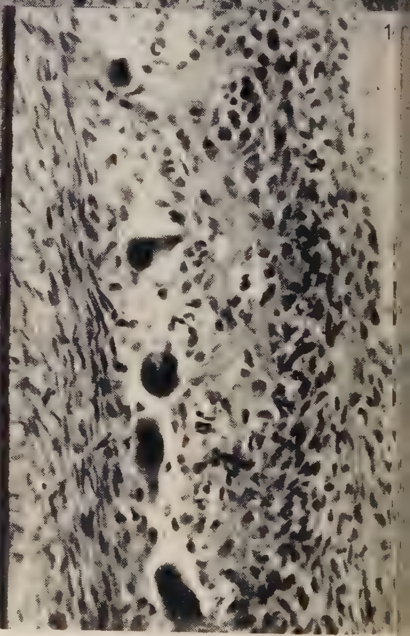
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HOYTE—Alizarin as an indicator of bone growth

JOHN KIRK

(*Facing p.* 443)

IN MEMORIAM

JOHN KIRK, M.B., CH.B., F.R.C.S.

Professor Emeritus John Kirk died in London on 26 September 1959, aged 77. A son of the manse he was born on 27 November 1881, and educated at George Watson's College and Edinburgh University. As an undergraduate he made his mark, being medallist in practical anatomy and prizeman in surgery—a fitting prelude to his future career—while in 1903 he played in the University golf team and was awarded his Blue.

Having graduated M.B., Ch.B., in 1904, he was appointed house surgeon at the Mildmay Mission Hospital, Bethnal Green, London, where he stayed for two years, and thereafter from 1907 to 1928 he served as resident medical officer to the New Zealand Presbyterian Mission in Canton, South China. During these 21 years he had two periods of furlough in Britain both of which he turned to good account. In 1913–14 he was admitted F.R.C.S. Edinburgh, and also attended the University course on Diseases of Tropical Climates, gaining the class medal in that subject. In 1921–2 he was full-time Demonstrator of Anatomy in the University of Birmingham, and the way events then were moving in Canton this was an experience which was to prove invaluable. By that time much had happened in Canton since his advent to an area hostile to foreigners, where sanitation was unknown and disease rife. For operating theatre there had been a whitewashed room; for light, oil lamps; for sterilizer, a tin bath of boiling water. But the passage of the years had seen great things achieved. With funds which he personally raised in New Zealand a new hospital was built, followed by a Nurses' Training School. In 1914 he was appointed Lecturer in Operative and Clinical Surgery at the Kung Yee Medical College, Canton, but it soon became apparent to him that he had undertaken an impossible task. Dissection was forbidden by law and his students simply were not equipped to understand surgical teaching founded on a sound anatomical basis. This state of affairs was not remedied until 1922 when dissection was permitted by the Chinese Government. The following year John Kirk accepted the invitation to take charge of the Department of Anatomy at the Kung Yee Medical College, the new buildings of which had just been completed. Some years later the Kung Yee Medical College became the Medical Faculty of the National University of South China.

Throughout his time in Canton he took a very real interest in medical education, and during his last two years there acted as Chairman of the Council of Medical Education of the China Medical Association. In 1928 he returned to England and was appointed Demonstrator of Anatomy at University College, London, under the late Prof. Sir Grafton Elliot Smith, whose memory he held in the highest regard. Only one year after going to University College he was called upon to act as Sub-Dean of the Faculty of Medical Sciences, and twelve months later he was appointed Senior Demonstrator of Anatomy. In 1931 a further responsibility which fell to him was that of conducting the Primary Fellowship Course in Anatomy, while from 1933 he was Senior Tutor in the Faculties of Medicine, Science and Engineering.

All these duties he discharged with great zeal and when in 1937 Prof. Yeates—happily still alive at a great age—retired from the S.A. Courtauld Chair of Anatomy in the University of London tenable at the Middlesex Hospital Medical School, John Kirk was appointed in his place. At the Middlesex, J.K., as he was affectionately known, spent twelve very active years and on his retirement in 1949 the University conferred on him the title of Professor Emeritus. The outbreak of war barely two years after his appointment found him shouldering a heavy burden, for the preclinical side of the Medical School had to be evacuated, first to Bristol and later to Leeds. The labour involved was enormous; everyone concerned played his part, but beyond all question Prof. Kirk shouldered the main burden. It may be that compared with his China days the difficulties to be faced were as nothing, but whatever the reason those who worked with him at that time will forever marvel at what he accomplished. As Sub-Dean of the Preclinical School he discharged the many duties of that office with consummate tact and characteristic thoroughness, the end of a long day in his own department meaning simply the start of an evening devoted to matters of School administration. The return to London before the end of the war brought fresh problems, for the V weapons were still to make their unwelcome appearance. Again he was to the fore, and the *modus vivendi* arrived at in the School owed much to his counsel. Throughout his retirement he maintained his deep interest in all the activities of the School and Hospital. For some years he served on the Board of Governors of the Hospital, while to the end he was a member of the Board of Studies in Human Anatomy of the University of London.

In 1952 the General Medical Council recognized his deep interest in medical education and invited him to act as their Visitor to Departments of Anatomy in Great Britain and Ireland. This was a heavy task, but one immensely to his liking. Every Department of Anatomy in the British Isles was visited on two occasions, once to inspect the general organization for teaching and research and on the second to attend as Visitor at the professional examination. The reports of these visits were full and accurate and of great assistance both to the General Medical Council and to the various Departments of Anatomy.

John Kirk was a member of our Society for more than thirty years and he served as Treasurer (1937–46) and as Vice-President (1947–49). Always a regular supporter of the Society's activities he continued to attend its scientific meetings throughout his retirement.

In the world of Anatomy Prof. Kirk will be remembered best as a truly magnificent teacher whether of the undergraduate or of the candidate for the Primary Fellowship Examination. His classes for the Fellowship, held first at University College and later at the Middlesex, attracted far more applicants than could be accepted and won for him a host of friends all over the world.

To write of John Kirk the man is not difficult for, quite simply, he was what he seemed—the happiest and kindest of men, filled with a deep and warm-hearted humanity whose serenity stemmed from an unswerving reliance on the Christian Faith. Himself he drove hard but to others he was the soul of consideration. Those who were privileged to work with him, staff and students alike, gained from him much more than a knowledge of anatomy, and his influence will go with them for the rest of their lives.

To Mrs Kirk and to his sons and daughter, for whom his death is not the end but the beginning, may we say that in abundant measure we share their gratitude for his life.

E. W. WALLS

REVIEWS

The Innervation of Muscle, a Biopsy Study. By C. Coërs and A. L. Woolf. (Pp. 149; 120 references; 281 figures; 42*s.*) Oxford: Blackwell's Scientific Publications, 1959.

This beautifully produced book is based on a study of over 450 biopsies. The appearance of the terminal pattern of motor innervation and the subneural apparatus in normal and pathological states is described and illustrated lavishly. Examples are taken only from cases with firmly established diagnoses. These include diseases of the anterior horn cell; lesions of nerve roots, spinal nerves and main nerve trunks; disorders of contractility; primary diseases of muscle fibres; diseases of the upper motor neurone and Parkinsonism. The diagnostic and prognostic value of the findings is discussed.

This book is of special interest to neuropathologists and to all who are interested in the inter-relationship of nerve and striated muscle fibres. It is to be regarded as an interim report, indeed one of the main purposes of the authors is to stimulate other observers, and in this they succeed admirably and deserve the warmest congratulations.

The techniques of taking the biopsies; the authors' own modifications of vital staining with methylene blue, of Bielschowsky silver stain and of the Koelle and Friedenwald stain for the subneural apparatus are described with full practical details. (In the latter, 2 hours preliminary treatment in physiological saline is advocated, but in the reviewer's hands the results have been disappointing in comparison with those obtained after immediate fixation.) The preparation of material for electron microscopy is also described, and the findings are to be published later.

The cholinesterase stain followed by Bielschowsky silver demonstrates the relationship between terminal arborizations of axons and the subneural apparatus. On the basis of such preparations the authors suggest that the 'flower spray' endings on intrafusal muscle fibres are motor in function. They also state that the fibres in limb and trunk muscles seldom have more than one end-plate. However, the physical limitations of histological methods, coupled with strong electrophysiological evidence to the contrary in both instances, suggests that their interpretations should be treated with caution.

The brief section on the development of the myoneural junction is disappointing and makes no reference to earlier work. It is in this section that the failure to name the muscle studied is regrettable in view of the gradient of development of the end-plate noted by Hewer (1935, *J. Anat., Lond.*, 69, 370).

The bibliography contains many references to recent work, but there are some surprising omissions, for example, there is no mention of the work of Cooper and Daniel on muscle spindles.

The preparations depicted are most elegant, and we are much indebted to the authors for a stimulating contribution to knowledge and for their generosity in publicizing the details of their techniques.

RUTH E. M. BOWDEN

Strong and Elwyn's Human Neuroanatomy. By Raymond C. Truex. 4th ed. (Pp. xiii + 511; 363 illustrations; £4. 0*s.* 0*d.*) London: Baillière, Tindall and Cox, Ltd. 1959.

That this book has reached its fourth edition is a sufficient recommendation in itself. For the first time neither of the two founder authors has contributed to this edition. Among alterations which have been made is the inclusion of a chapter devoted solely to the rhinencephalon. This provides a concise account in traditional terms of a somewhat controversial and awkward topic worthy, however, of the individual attention it has now been given. Praisworthy emphasis has also been placed upon the appreciation of the gross structure and blood supply of the brain and spinal cord by additions to this section. The text matter covers the whole subject in sufficient detail to be adequate for advanced students whose knowledge may be supplemented by using the adequate bibliography. One unusual but valuable feature of the book is the inclusion of a chapter on the segmental and peripheral innervation.

The book contains a profuse number of illustrations, thirty-seven of which are new to this edition and of these the diagrams illustrating lesions in the spinal cord should prove of great help in understanding a difficult section. It must be admitted, however, that some of the illustrations would bear improvement, but this should not detract from the excellence of others; particularly, the Weigert preparations of the spinal cord in which the cell columns are schematically indicated, and the exploded wiring diagrams of the pathways in the central nervous system.

The student of Neuroanatomy is faced with a difficult task when selecting a text-book from the many available; he should have no cause for regret should this be the text-book of his choice.

W. HEWITT

The Cell of Schwann. By GILBERT CAUSEY. (Pp. xi+120; 21*s*.) Edinburgh: E. and S. Livingstone Ltd.

It was largely from his studies on the nervous system that Theodor Schwann reached his conclusion that 'the similar cellular origin is common to everything living'. It is strangely fascinating to see how, from studies of the apparently secondary satellite cells that bear his name, discoveries of fundamental features of life are still being made. The periodicity of the myelin that the Schwann cell makes is sufficiently marked to allow analysis of it by X-ray diffraction and since electron microscopy shows that it is made of many layers of cell surface we can argue back to the structure of the cell membrane. Moreover, since similar membranes occur throughout the cell, often in continuity with the surface, we reach what we may call the neo-Schwannian view that the cell is nearly all membrane.

Prof. Causey has packed into a small space the essentials of the history of neurohistology. The method of arrangement of the material makes the book continually interesting and stimulating to read. He balances accounts of points of view of the last century with work of the last decade, and discusses in perspective such controversial problems as the 'ectodermal' nature of neurofibroblasts and the permeability of the sheaths that they produce. The place of histological investigations in study of the functioning and pathology of the nervous system is continually brought to mind. Indeed, anyone will find the book a stimulating introduction to many neurological problems. That expectation is often aroused but left unsatisfied is inevitable in so short a treatment. For example one would like to hear more of what Prof. Causey believes about incisures, and to have more and more high-resolution electron micrographs, for instance of nerve regeneration.

The book shows what can be done by a courageous survey, even of a rapidly growing field, keeping in mind a historical perspective. Perhaps Prof. Causey will venture to repeat the process on a wider field, and we may hope that he and Messrs Livingstone will then give us an index in addition to their otherwise excellent production.

J. Z. YOUNG

Der funktionelle Bau der Herzkammern. By ALEXANDER PUFF. (Pp. vi+87; 80 illustrations, Kartoniert DM. 18.) Stuttgart: Georg Thieme Verlag. 1960. (A publication from the series of Zwanglose Abhandlungen aus dem Gebiet der normalen und pathologischen Anatomie, Heft 8, edited by Prof. Bargmann and Prof. Doerr of Kiel.)

This book deals with the muscular architecture of the ventricles and kinetic analysis of the motion of the heart. It is based on human and animal material. In addition to his own findings the author gives a comprehensive survey of the current and old literature. He deals in detail with the fibre systems of the ventricles and especially the papillary muscles. In order to analyse the motion of the heart under normal and experimental conditions he employs high speed cinematography (500 and 1000 frames/sec.). The motion of the heart and the curve of its electrocardiogram are filmed simultaneously and appear side by side on each frame.

The book is excellently produced, the style is attractive, and the illustrations are of a very high standard. The bibliography contains over one hundred references. The book can be warmly recommended to anatomists and to clinicians interested in the function of the heart.

N. CAUNA

BOOKS RECEIVED

The Sexual Cycle of Vertebrates. By J. F. D. Frazer, 1959. (Pp. 168; 6 figs. 10*s*. 6*d*.) London: Hutchinson University Library.

Basic Anatomy and Psychology. By H. G. Q. Rowett, 1959. (Pp. 124. 11*s*.) London: John Murray.

Natural Selection and Heredity. By P. M. Sheppard, 1959. (Pp. 212; 8 figs. 10*s*. 6*d*.) London: Hutchinson University Library.

Grundriss der normalen Histologie und mikroskopischen Anatomie. By H. Voss, 1959. (Pp. v+221; 206 figs., 2 plates. DM. 13.) Leipzig: Georg Thieme.

Leitfaden der mikroskopischen Technik. By H. Haug, 1959. (Pp. v+218; 57 figs., 2 plates. DM. 29.80.) Stuttgart: Georg Thieme.

Folia Biologica. Chief editor, I. Malek, Vol. 5, Fasc. 6, 1959. (Kcs. 12.50.) Prague.

Russian Review of Biology. English editor, H. Munro Fox, Vol. 48, No. 1, July–Aug. 1959. (£9 for first nine issues, single issues, £1. 10*s*.) Edinburgh: Oliver and Boyd.

Les Nouveaux Termes Anatomiques. By G. Olivier, 1959. (Pp. 146, 800 Fr.) Paris: Vigot Fréres.

Beitrgäe zur vergleichenden Morphologie des Nagetiergehirnes. By G. Pilleri, 1959. (Pp. iv+124; 54 figs. S.Fr. 22.) Basel: S. Karger.

Journal of Reproduction and Fertility. Edited by C. R. Austin, 1960, Vol. 1, no. 1. (24*s*. per number.) Oxford: Blackwell Scientific Publications.

A Stereoscopic Atlas of the Dog's Brain. By R. K. S. Lim, C. Liu and R. L. Moffitt, 1960. (Pp. v+93; 68 figs. 74*s*.) Oxford: Blackwell Scientific Publications.

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

FEBRUARY 1960

An Ordinary Meeting of the Society for the Session 1959–60 was held on Friday, 26 February, at the Anatomy Department, Charing Cross Hospital Medical School, W.C. 2. The President (Prof. J. D. Boyd) occupied the chair.

The following are the authors' abstracts of the papers presented.

Preliminary observations on the implantation of rabbit blastocysts in vitro.

By T. W. Glenister. *Charing Cross Hospital Medical School, London*

Blastocysts obtained 7 days after mating, from which the zona pellucida had been dissected off, were explanted on strips of maternal endometrium. These combined explants were incubated as organ-cultures for periods of up to five days, in a medium containing maternal serum. Within 72 hr., a proportion of the blastocysts are found to be attached to the endometrium. The material has been sectioned serially and reveals that the blastocysts have become connected to the endometrium by pedicles of invasive cellular trophoblast emanating from the vicinity of the animal pole of the blastocyst. The site at which the endometrium is being invaded by trophoblast is denuded of uterine epithelium, but the latter may on occasion be traced right up to the margin of the invasive pedicle. Propinquity to other blastocysts on the same endometrial strip does not appear to affect the tendency to implant. A preliminary series of over three dozen attempts to obtain implantation in culture has resulted in a success rate of about one in three.

It is suggested that this method will provide a means of studying in a controlled medium the factors concerned in implantation and in the control of the growth, differentiation and invasiveness of trophoblast.

The trophoblastic cell-islands of the human placenta.

By J. C. Coleman, *Charing Cross Hospital Medical School, London*

In an attempt to elucidate the topographical relationships existing between placental cell-islands and villi, placentae covering a wide age spectrum, fixed *in situ*, and serially sectioned were examined.

The islands are found to be elongated fusiform masses to which the villi are closely related over the entire length of the island. This finding was confirmed by graphic reconstructions, which, due to technical difficulties, were confined to two 10 mm. specimens.

Some villi lose the syncytiotrophoblast over the area in contact with the islands. In these circumstances a gradual transition of cell-types from the basophil, cuboidal cells of the Langhans' layer to the paler, polygonal cells of the islands is observed. The occurrence of mitoses, both in the basal Langhans' layer, and in the cellular mass on the insular surface of the Langhans' layer is taken to indicate that cells are proliferated from this area into the island. These formations, of which there may be from four to six in each island, are termed trophoblastic eruptions. The appearance of these eruptions suggests that, at the 10 mm. stage, the islands receive a major contribution to their cellular component from contiguous villi.

On the probable genetic character of human placental types, with some remarks on the structure of placental cotyledons.

By P. Bacsich and J. M. Crawford. *University of Glasgow*

It is known (Bacsich & Smout, *J. Anat., Lond.*, **72**, 1938) that, according to the pattern of the foetal arteries running in the chorionic plate, all human placentae fall into two distinct groups: (1) the 'disperse' type, in which the oncoming umbilical arteries undergo a succession of dichotomous divisions, rapidly diminish in calibre and resemble in their arrangement the spokes of a wheel; (2) the 'magistral' type, where the main arteries, giving off mostly small side branches, almost reach the margin of the placenta before there is any marked reduction in their size.

The present communication represents the results of two separate lines of inquiry:

(1) During the past 20 years a number of twin pregnancies have been examined and the two placentae, even in the case of non-identical twins, always belonged to the same type. In a further series, placentae of both first and second pregnancies were studied and it was found that in the second pregnancy the placental type of the first pregnancy was invariably repeated.

(2) As a sequel to studies on the finer morphology and capillary circulation of full-term and foetal placentae (Crawford, *J. Obstet Gynaec.* **62**, 1955; **63**, 1956; **70**, 1960), we were able to observe that the placental type is already recognizable at the 12th week of pregnancy. Moreover, it became apparent that the characteristics governing placental type are not confined to the vessels of the chorionic plate alone, but also influence the basic morphology of the cotyledons. Cotyledons of the magistral type of placentae are bushy, with numerous brush-like villi emerging from a common stem, while those of the disperse type are less well developed and the much smaller number of villi show frequent dichotomous divisions.

The theory has been advanced (Schordania, *Arch. Gynaek.* **153**, 1929), probably on insufficient evidence, that the vascular pattern of a placenta is not a haphazard phenomenon, but is genetically determined by the vascular characteristics of the mother. The authors believe that the above observations lend additional support to this assumption.

Experimental study of the testicular descent.

By K. M. Backhouse.
Charing Cross Hospital Medical School, London

A reappraisal of experimental studies so far carried out on testicular descent and an analysis of the optimum times when experimental studies could be carried out on the gubernaculum testis has been made.

It has been shown that testicular descent, bringing the testis into the position of the sexually inactive animal, takes place in all Eutherian mammals examined before birth, though the process (cf. rat) may not be completed until after birth. Testicular descent of puberty in those animals exhibiting such a phenomenon is essentially one of scrotal hypertrophy, a very different process from true foetal testicular descent. Experimental studies carried out on postnatal Eutherian mammals do not influence the gubernaculum testis or true testicular descent. In order to do this, studies must be made on foetal stages in Eutherian mammals or early pouch young stages of marsupials.

A histochemical study of phosphorylase in embryonic tissues.

By T. Adesanya I. Grillo. *University of Cambridge*

A parallel study has been made of glycogen and phosphorylase in the liver, skeletal muscle, heart and cartilage of chick embryos. Glycogen was studied by the PAS and sulphation methods. Phosphorylase was demonstrated by the histochemical method of Takeuchi and Kuriaki (*J. Histochem. Cytochem.* **3**, 153–160), suitably modified to give satisfactory results with liver. In the tissues studied, phosphorylase could not be detected until after glycogen was already present. In cardiac muscle and liver the enzyme appeared 1 or 2 days after the carbohydrate. In skeletal muscle the interval was much greater: whereas glycogen was present by the 4th or 5th day, phosphorylase could not be demonstrated until the 14th day. It therefore appears that embryonic glycogenesis initially occurs without appreciable phosphorylase activity, though this enzyme may later be important for the breakdown of glycogen.

The demonstration of erythrophile substances in tissues. By E. Gurr and M. A. MacConaill. *University College, Cork*

This work is a continuation of that described to the Society at its last meeting 27 November. It has long been known that orange G has a marked obliterative effect on many stains. When this dye (0·5 % aqueous) is applied to specimens which have been stained in acidified acid fuchsin, followed by dilute light green, the colours of the tissues disappear. If, however, the sections are given a bath of acetic acid (2 %) before treatment with orange G then the erythrophile (fuchsinophile) elements remain red according to the degree in which they have combined with the acid fuchsin originally. By this technique very clear pictures can be obtained of the capillary and venous elements of a tissue by means of the stained erythrocytes, the sections being particularly suitable for examination by polarized light and/or phase-contrast. It appears that the two-stage FALG process indicated above is better than the one-stage MG Blue process for this purpose. It is also much better than simple staining with acidified acid fuchsin. This method, like the FALG methods themselves, enable mitotic nuclei to be picked out under as low a power as 10 × objective by the erythrophilia of the karyoplasm. The chromosomes, stained dark blue, stand out clearly under higher powers. Some chemical features of these reactions were discussed.

The vascular canals of the condylar cartilage of the mandible. By H. J. J. Blackwood. *Royal Dental Hospital, London*

Vascular canals first appear in the growing condylar cartilage of the mandible about the 130–150 mm. C.R. stage of development. The number of canals in each cartilage increases rapidly during intra-uterine life and during the first few months of postnatal life but thereafter the canals tend to decrease in number and finally disappear during the 3rd year of life. The canals are formed by the inclusion within the cartilage of columns of undifferentiated cells from the perichondrium and following their inclusion they rapidly become vascularized from the medullary cavity of the mandible. The blood vessels enter the free extremities of the canals in the medullary cavity and course upwards towards the articular surface of the cartilage where they ramify in the transitional zone of cells; some vessels enter the fibrous articular layer and so come to lie very close to the joint space. Within the cartilage some vessels form short anastomotic loops with adjacent vessels while others anastomose with vessels from adjacent canals. It is assumed that the function of the vascular canals is to aid nutrition and interstitial growth of the cartilage, especially during the first few years of life when there is a demand for rapid growth.

Observations on columnar and goblet cells of the small and large intestine. By B. A. Young and F. R. Johnson. *London Hospital Medical College, London*

In the present investigation electron microscopy of columnar cells of the intestine has confirmed that the striated border is formed of regularly arranged microvilli composed of central dense cores with surrounding membranes. The central cores of the microvilli extend into the apical cytoplasm where their extremities terminate in association with electron dense bodies. An examination of the intestines of different species (man, monkey, cat, rat, rabbit and seal) has revealed that there are only minor structural variations in the striated border from one species to another and between the small and large intestine in the same species. A study of globlet cells during the resting and early filling stages of activity indicates that these cells also have a striated border which is similar to that of columnar cells. The appearance of columnar and goblet cells during development in man and rat was described.

Cell number in the anuran ventral horn. By A. F. Hughes and J. A. F. Fozzard.

University of Cambridge

In early hind-limb bud stages of the tadpole of *Xenopus laevis*, the lumbar ventral horn becomes a discrete group of neuroblasts containing about 3000 cells. This number is reduced to about 1000 by the period of metamorphosis, a number which is also found in the adult animal. Reduction in total cell number is accompanied by cell degeneration, which is especially intense at the time when the neuroblasts are most rapidly maturing.

Irradiation at early hind-limb stages with X-rays at a dosage (40–60 r. units) below that which evokes wholesale destruction of neuroblasts, slows the general development of the tadpole, but that of the ventral horn is more severely retarded than the growth of the hind-limbs. Under these circumstances, degeneration within the ventral horn is much reduced for a fortnight or more, but subsequently, both a wave of cell degeneration and the phase of maturation of the neurones occur together at a period later than usual in development. Thus these two histogenetic features remain associated.

If a hind-limb 3·0 mm. long is amputated no regeneration occurs before metamorphosis. During the period 36–48 hr after ablation, the number of ventral horn cells on the operated side falls temporarily by several hundreds, with accompanying cell degeneration. On the 3rd day, the normal number is restored, and remains equal on both sides until metamorphosis. It thus seems that once the ventral horn has differentiated in the first instance, the control of cell number therein does not directly depend on the immediate influence of the limb.

The thalamic projection in *Lacerta viridis*. By T. P. S. Powell and L. Kruger.

University of Oxford

An experimental study has been made of the telencephalic projection of the nuclei of the thalamus of *Lacerta viridis* using the method of retrograde cell degeneration. Following complete removal of the telencephalon severe cell loss is seen in the nucleus rotundus and cell shrinkage in the adjacent part of the nucleus dorsomedialis anterior. From an analysis of the degeneration after more localized lesions it has been found that these nuclei project upon the lateral part of the palaeostriatum. Sections of the same experimental material stained with the Bodian technique have shown that removal of the telencephalon results in severe degeneration of the anterior and intermediate thalamic components of the lateral forebrain bundle. In experiments with smaller lesions it was found that the intermediate thalamic component degenerated only after involvement of the palaeostriatum, and that the anterior thalamic component was related to both the dorsal striatum and the palaeostriatum. It may be concluded that the nucleus rotundus and the nucleus dorsomedialis anterior constitute the total thalamic projection upon the telencephalon, that the fibres from these nuclei pass in the intermediate thalamic component of the lateral forebrain bundle, and that the anterior thalamic component is composed of fibres descending from the dorsal striatum and the palaeostriatum.

Qualitative and quantitative studies of the myenteric plexus in the small intestine of the cat. By D. B. Leaming and N. Cauna. *Departments of Surgery and Anatomy, King's College, Newcastle-upon-Tyne*

Myenteric plexuses of duodenum and jejunum have been investigated in twenty-seven adult cats by a simplified Bielschowsky–Gross silver impregnation method and Koelle's histochemical technique for cholinesterase activity (pH 5·3).

It was found that the plexus was situated 50–150 μ deep to the peritoneal surface of the gut. There were approximately twice as many nerve cells in the mesenteric as in the antimesenteric zones, but the distance between cells did not exceed 2 mm.

In duodenum and jejunum, approximately one-third of all nerve cells stained deeply with silver; the remaining two-thirds stained lightly or remained unstained. In the upper jejunum, there were 10·6 deeply staining nerve cells per mm^2. in an area of 93·3 mm.^{2}. A sample count in the duodenum near the mesenteric border showed 43 deeply staining and 79 lightly staining nerve cells per mm.^{2}.

After incubation with acetyl substrate two types of nerve cells were found—cholinesterase positive and cholinesterase negative. Incubated with butyryl substrate, all nerve cells were negative. Tissues surrounding the nerve cells gave a positive reaction with both substrates.

The histochemical reaction of the myenteric plexus was found to be similar to that of the sympathetic ganglia.

Observations on the myenteric ganglion cells in congenital pyloric stenosis.

By J. R. Rintoul and N. F. Kirkman. *University of Manchester*

Thirty-two biopsy specimens of the pylorus from cases of congenital hypertrophic stenosis (together with one normal biopsy specimen obtained at operation and eight specimens secured at necropsy) have been examined by routine histological staining procedures and by specific nerve staining techniques.

Recent work (Nielsen O.S., *Acta paediat.*, 1956, **45**, 636) has suggested that degeneration of the myenteric ganglion cells is the principal aetiological factor in this condition. We are unable to corroborate this finding. Microscopical appearances suggestive of autolytic change were evident in some of the autopsy specimens, but signs of degenerative change were not observed in biopsy specimens stained by nerve staining methods.

Employing cresyl violet, gallocyanin, and H. and E. staining methods, appearances resembling cloudy swelling were obtained on occasions in the biopsy specimens, but in no instance was disappearance or fragmentation of the nucleoli, nucleus or nuclear membrane observed. Since the results obtained by the latter methods are somewhat equivocal we believe that it is unwise to draw conclusions from such evidence alone regarding the aetiology of infantile hypertrophic pyloric stenosis.

Observations on the innervation of the rectum and anal canal.

By Anne M. Holmes. *University of Manchester*

Methylene blue, silver, Champy-Coujard and gold chloride staining methods have been employed in innervation studies of the rectum and anal canal in the human, monkey, cat, rabbit, guinea-pig and rat. The submucous plexus, found throughout the whole of the rectum and anal canal, gives rise in its proximal part to a periglandular plexus which surrounds the bases of the tubular glands. In the pecten, sensory corpuscles, a subepithelial plexus and intra-epithelial nerve fibres may be seen; whilst below the level of Hilton's white line only free nerve endings, nerves related to hair follicles and intra-epithelial nerves are found as sensory conductors. Neurons are found in the submucous plexus only as far distal as the pectinate line, whereas in the myenteric plexus (which ends by sending fibre bundles both through and distal to the internal sphincter to join the submucous plexus) they may be found in the pecten.

These findings lend support to that view of the development of the anal canal which places the junction of proctodaeum and cloaca at the level of the anal valves.

Glycogen fractions in the conducting system of the ox heart.

By W. J. C. Wilkinson, J. H. Kugler and C. N. Eccles. *University of Sheffield*

Glycogen is known to disappear very quickly in myocardium but F. Davies (personal communication) noted that glycogen could be detected histochemically in the conducting system of the ox heart long after it had disappeared from the normal heart muscle, and previous personal work has shown that present day histochemical techniques for the demonstration of tissue glycogen depend upon the acid soluble glycogen that is present in the tissues. Preliminary experiments suggest that this paradoxical loss of glycogen in myocardium and not in the Purkinje fibre is due to the relative amounts of glycogen that are present in both tissues. In the present work both the total glycogen and the acid soluble glycogen content of the conducting system and the myocardium has been determined and correlated with the histochemical picture. The physiological implications of these results were discussed.

Weights of abductor and adductor muscles of the human larynx.

By J. L. Withington. *Royal Free Hospital School of Medicine, London*

In an attempt to evaluate Semon's Law, which states that the abductor muscles of the larynx are more prone to paralysis than the adductors in partial damage to the recurrent laryngeal nerve, attention was drawn to the weight of the two groups of muscles. Most accounts state that the adductors greatly outweigh the abductors and two state that they are equal in weight. Few quantitative measurements appear to have been made.

In a series of twelve human larynges the weight of the abductor muscles was less than 25 % of that of the adductors and this relationship bore a high coefficient of correlation. It is suggested that this inequality in bulk between the two groups of muscles might contribute to the greater vulnerability of the abductors in partial injury to the recurrent laryngeal nerve.

Digital rotation. ByK. M. Backhouse. *Charing Cross Hospital Medical School, London*

The role of digital rotation has been examined and its mechanism studied experimentally. It is essentially a movement used with metacarpophalangeal flexion and deviation, i.e. fine digital movements. As such, it is a movement of the interosseous rather than lumbrical muscle. This contention has been examined by stimulation and electromyography, using needle electrodes in each case. Although digital rotation of the index and middle fingers is produced in median nerve block at the wrist, apparently due to loss of the lumbrical muscles to these digits, a similar deformity can be produced by a local anaesthetic in the metacarpophalangeal joints. An alternative explanation of the phenomenon could therefore be loss of metacarpophalangeal joint sense, leading to imbalance in the interosseous muscles.

The effect of female sex hormones on melanin pigmentation. By R. S. Snell.

King's College, Newcastle-upon-Tyne

A histochemical investigation into the effect of these hormones on the activity of the melanocytes in the skin was carried out on eighteen mature female guinea-pigs. The animals were ovariectomized to remove any side effects which might have been produced by the cyclical activity of the ovaries and one month later skin biopsies were taken from the ear, anterior abdominal wall and areola. Six of the animals then received a four week course of oestrogen injections, six received a similar course of progesterone and the remaining six received a course of oestrogen and progesterone given together. After the animals were killed a further series of skin specimens were removed from areas adjacent to those taken previously. All skin specimens were processed with the Dopa reagent.

In all the regions oestrogen produced an increase in the amount of melanin both inside and outside the melanocytes and the effect was greatest in the skin of the areola. Progesterone had a similar but less marked effect on melanogenesis. Oestrogen and progesterone when given together stimulated melanogenesis to a far greater degree than when either hormone was given alone. The significance of these findings was discussed.

Quantitative studies of the healing of immobile skin. By J. Joseph and Jane Townsend. *Guys' Hospital Medical School, London*

An area of skin 20 × 20 mm. was removed from the inside of both ears of rabbits, indian ink marks were made 1 mm. from the edge of the denuded area at the corners and the middle of each edge and the ears were dressed with penicillin, tulle gras, surgical gauze and plaster of Paris. The dressings were removed after 5 or 10 days and specimens after 5, 10, 15, 20, 30, 40, 60, 100 and 150 days were obtained. The growth of new epidermis and the extent of the remaining denuded area, if any, were measured microscopically at intervals of 200μ. These measurements were charted on squared paper at 10 × magnification and the areas covered by new epidermis and the remaining denuded areas were estimated from the charts. The macroscopic appearance of the healing area was recorded at frequent intervals by photography. The qualitative changes of the new epidermis and its underlying tissue were also studied.

It was found that the denuded area was covered by spreading epithelium from the edge and not by contracture of the surrounding skin. This was clearly shown by the fact that the distance between the Indian ink dots on the middle of the edges of the denuded area remained the same throughout the period of growth of the epidermis. After 5 days the denuded area was reduced by about 25 %, after 10 days by 61 %, after 15–20 days by 95 %, and after 30 days by 98 %. All the specimens of 40 days' duration or more were completely covered with epidermis. It was estimated that the epidermis grew about 0·86 mm. in the first 5 days and about 2·70 mm. in the first 10 days. For reasons discussed it was not regarded as useful to estimate this growth after 15 or more days.

Some of the more striking qualitative changes in the tissue covering the denuded area were mentioned, e.g. the peculiar regrowth of hair, the presence of mitotic figures in the new epidermis before the denuded area was covered, the formation of a new 'dermis' etc.

In vitro studies of the interaction of immune lymph node and homologous tissue. By R. J. Scothorne and I. Nagy. *University of Glasgow*

In view of the well recognized role of lymphoid organs in the destruction of homografted tissue, it was decided to investigate the interaction between lymph node and homologous tissue *in vitro*.

Grafts of tail skin from donor CBA mice were made to the chest wall of recipient Swiss white mice. At intervals thereafter the regional lymph nodes draining the graft were removed and cultured in intimate side-by-side contact with lymph node fragments from the original donor. The organ culture technique and the protein-free synthetic medium T8 of Trowell (1959) were used.

After 4 days *in vitro* the alien lymph node fragments have become fused together and, despite this intimate contact of donor cells and the 'immune' lymphoid cells of the recipient both appear entirely healthy. This failure to reproduce the homograft reaction *in vitro* is discussed in relation to previous attempts by other workers and to the general problem of homograft immunity.

Some observations on the response of lymphocytes to immunization. By K. W. Keohane and W. K. Metcalf, *University of Bristol*

The Refractive Index of the cytoplasm of small lymphocytes obtained from various sites in Wistar albino rats has been measured by suspension in isotonic bovine serum albumin solutions of various refractive indices. The refractive index of the solution which was the best match for the small lymphocyte cytoplasm, as determined with the phase contrast microscope, was measured with an Abbé Refractometer.

It was found that lymphocytes, whatever their course, had the same cytoplasmic refractive index in normal animals (1·3549 ± 5). A batch of rats suffering from rat pneumonia, a chronic infection, proved to have a significantly raised refractive index in the cytoplasm of their small lymphocytes, particularly in those from the lymph nodes (1·3580 ± 6). A similar rise followed immunization of normal rats within 72 hr. The details of this response have been followed for 3 weeks after the first injection.

The reasons for believing that the refractive index is essentially determined by protein concentration were given and the significance of the changes during immunization discussed.

Changes in the first regional lymph node after homografts and autografts of cancellous bone. By R. G. Burwell. *University of Leeds*

The reaction of the regional lymph node to a constant weight of *fresh* homogenous or autogenous cancellous bone, removed from the iliac crest and inserted into the subcutaneous tissue of the ear, has been investigated in seventy adult male rabbits.

The increase in weight of the first regional node of the side receiving the graft is considerably greater in the homografted than in the autografted animals. After a homograft, a characteristic cellular response occurs in the local lymph node, wherein great numbers of pyroninophilic 'large lymphoid cells' appear in the diffuse lymphoid tissue of the cortex and in the medullary cords. This finding is in keeping with the response of the first regional nodes to skin homografts (Scothorne, R. J. & McGregor, I. A. *J. Anat., Lond.*, 1955, **89**, 283–292), and to 'second-set' homografts and 'first-set' heterografts of cartilage (Craigmyle, M. B. L., *J. Anat., Lond.*, 1958, **92**, 74–83).

Further analysis of the nodes reacting to bone homografts has revealed a *sectoral distribution* of these large lymphoid cells. This localization of function in the node, is probably determined by the points at which the lymphatic drainage from the implant enter the node.

A quantitative analysis of the large lymphoid cell response has been attempted in *the reactive sectors of the diffuse lymphoid tissue* of the cortex at intervals of time up to 14 days after insertion of the graft. The mean number of large lymphoid cells per unit area of this part of the cortex has been found to rise rapidly and reach a maximum between the 4th and 6th days after homografting; a more gradual decline then ensues, the count returning to a normal level by the 14th day after insertion of the foreign graft.

STUDIES ON THE STRUCTURE OF AUTONOMIC NERVES IN THE SMALL INTESTINE, CORRELATING THE SILVER-IMPREGNATED IMAGE IN LIGHT MICROSCOPY WITH THE PERMANGANATE-FIXED ULTRASTRUCTURE IN ELECTRONMICROSCOPY*

By K. C. RICHARDSON

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Progress in the study of the autonomic innervation of organs such as the small intestine has been slow during the past decades, principally because the precision necessary to reveal nervous pathways and interconnexions in the tissues is difficult to obtain by the conventional methods of light microscopy. Until recently, in fact, direct comparisons between the structural appearance of living, non-myelinated nerves and their fixed and stained counterparts have been lacking. Weddell & Zander (1950, 1951) have made a critical study of non-myelinated nerves in the living cornea stained *in situ* with methylene blue, and in the isolated, unstained cornea by phase-contrast microscopy. They have been able to establish a standard for the living nerves, and so to recognize the various forms of distortion produced by post-mortem changes, degeneration following lesions or accidental mishandling of the tissues, as well as the often gross distortion produced by fixation in formalin and staining. It is possible indeed from this work to take the extreme attitude that non-myelinated nerves should be studied only in the living state if a reliable account of their morphology is to be recorded. They are remarkably labile structures. At the same time it is clear why so many different interpretations exist of the structure of fine autonomic nerves in regions of the body where no direct observation of the living tissues can be undertaken. Electronmicroscopy offers a new approach to the problem of autonomic innervation in that the methods of fixation are known to involve much less distortion than the conventional methods of neuro-histology. The increased magnification, moreover, allows one to identify neurites, Schwann cells, synaptic endings and other components with much greater accuracy. Thus Gasser (1952), Hess (1956), Robertson (1959), Elfvin (1958) and others have described the ultrastructure of autonomic nerve bundles in various species. The very limited amount of material which can be examined by electronmicroscopy, however, still makes it essential to use the methods of light microscopy if we wish to build up a three-dimensional picture of nerve distributions and their interconnexions. What is needed is a reliable method of fixation and staining for light microscopy which will show fine autonomic nerves in a condition comparable with what we know of their ultrastructure and fitting as closely as possible to the appearance of non-myelinated nerves in a living tissue such

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as the cornea. Intravitam staining with methylene blue has served its purpose well in the past for mapping out the general distribution of autonomic innervation, but there are many objections to its use, particularly the poor fixation following staining. Much greater specificity in the identification of autonomic nerves is obtained with the cholinesterase technique of Koelle & Friedenwald (1949), but again the fine structural details are marred by indifferent fixation.

Using the known ultrastructure of the intestinal nerves as a standard it was decided to investigate whether a method of silver impregnation could be devised which would be reliable in operation and give a more faithful picture of the autonomic ground plexus (AGP) within the limits of fixation and optical resolution. Unless this could be done there could be little confidence in results obtained by silver staining from experimental material.

Accordingly, this paper consists of three parts. In the first part a short description is given of the structural organization of the nervous tissue of the intestinal wall, with special reference to the fine structure of the AGP supplying the muscle coats. Then the technique of silver impregnation is formally presented, analysed and discussed, with emphasis on the limits within which the technique can be relied upon to give optimal results. The second part is concerned with validating the silver technique by comparison with results obtained by electronmicroscopy. In the third part the results obtained are discussed, and examples from previous authors are criticized or re-interpreted in the light of the present findings.

MATERIAL AND METHODS

The material was taken from adult rabbits of mixed strains, supplemented by a limited amount of adult cat intestine for some of the silver impregnation experiments. For electronmicroscopy a modification of the potassium permanganate fixation of Luft (1956) was used because during fixation the ganglia and major bundles of Auerbach's plexus were stained more strongly than the surrounding muscle. This made it possible to isolate individual ganglia or nerve bundles for cutting in a pre-selected plane. A detailed description of the permanganate fixation will be given in another paper. After fixation the material encased in 2% agar was dehydrated and embedded in Araldite according to a method described elsewhere (Richardson, Jarett & Finke, 1960). Ultra-thin sections were mounted on collodion-coated copper mesh grids and examined with RCA electronmicroscopes, models EMU 2B or 2E. The method of silver impregnation which arose from the experiments to be described has been summarized in a separate section below.

PART I

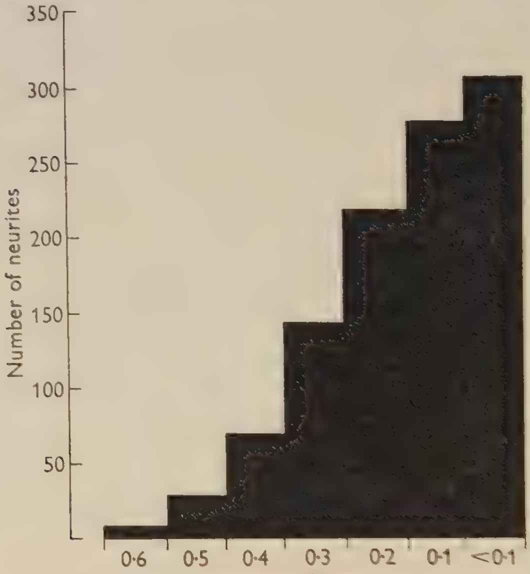
Preliminary observations

If the intestinal wall is reduced to a sheet of longitudinal muscle by removing all its internal layers, a heavy silver impregnation will show the components illustrated in Pl. 1, fig. 1, in the areas between the ganglia and primary bundles of Auerbach's plexus. Thus, against the background of muscle, one can distinguish secondary and tertiary nerve bundles, blood vessels and interstitial cells. These are held together by a fairly dense aggregation of reticular connective tissue fibres which remains completely unstained and is not brought into prominence even in electronmicrographs without some form of staining. From the tertiary plexuses, and to a lesser extent from the primary and secondary plexuses, very fine nerve bundles enter the adjacent tissue, or pass inwards with the blood vessels to the submucosa. Under the electronmicroscope the tertiary bundles in cross-section are found to consist of numerous small neurites (see Pl. 2, fig. 8) enveloped singly or in groups by the cytoplasm and cell membranes of Schwann cells. At the surface of each bundle the neurites are often incompletely surrounded by Schwann membrane and come directly into contact with the Schwann basement membrane which covers the entire surface of each bundle. Nearby the elongated processes of interstitial cells (see Pl. 2, fig. 8) interweave amongst the tertiary bundles, sometimes making what appear to be random contacts with the basement membrane.

Following the branches of the tertiary plexus into the muscle coats, the bundles of neurites become much smaller in diameter as they ramify in the spaces between the individual smooth muscle fibres. Many of the neurites lie close to the surface of the sheath which often envelops them in groups rather than insulating them individually. Occasionally some difficulty is experienced in identifying transverse sections of the processes of Schwann cells which, according to Elfvin (1958), interdigitate with one another to form the characteristic nucleated strands commonly regarded in the past as composed of a syncytium. Thus in Pl. 2, fig. 7, there are five discrete areas of Schwann cytoplasm bounded by membranes which reach up to the basement membrane. The area to the right of the bundle is almost divided into three.

A sample of fifty of these bundles in electronmicrographs has been examined in detail, estimating the numbers of neurites and their approximate diameters. The results are summarized in Text-fig. 1, from which it can be seen that the neurites range in diameter from $0{\cdot}6\,\mu$ to less than $0{\cdot}1\,\mu$. There were very few large neurites and over half the total number of neurites measured were $0{\cdot}1\,\mu$ or less in diameter. Such a result could be interpreted to indicate that each neurite bifurcates repeatedly to give branches which become progressively smaller in diameter, and that the smallest neurites may be those which happen to be sectioned near their terminations. However, if we take some of the smallest bundles (see Pl. 2, figs. 4–7) it will be seen that these contain thick neurites as well as an assortment of those of intermediate and small diameters. The problem still remains as to how we are to identify the often numerous cross-sectional profiles (see Pl. 2, fig. 6) which lie outside the basement of the neurite bundles, scattered in the spaces between the smooth muscle fibres. These are probably too numerous to be simply obliquely sectioned extensions of the muscle fibres, or pseudopodia of interstitial cells. It is difficult to believe that some are not neurites which have left their bundles to gain a closer relationship with the muscle fibres.

The foregoing structural details form the basis of our present understanding of the structure of the AGP and the problem is now posed as to how we are to reveal as much as possible of these details in a stained preparation. In a previous publication (Richardson, 1958). an investigation was reported of the intramural nervous tissue of the rabbit small intestine as seen by electronmicroscopy, and by methods of silver impregnation. It was realized at the time that although a reasonable correlation might be made between structures revealed by these two methods, there was much to be done in making these correlations more exact. For example, in Pl. 5, fig. 18, of the above paper the silver-stained AGP distributed in the circular muscle coat did not clearly show a system of parallel neurites invested by a Schwann sheath and accompanied by interstitial cells with branching processes. The electronmicrographs had revealed that these components were structurally quite distinct. In Pl. 1, figs. 3 and 4, of the Auerbach tertiary plexus with interstitial cells, the differences were more evident because these components were much more widely separated, but the optical appearance of the nerve bundles was far from satisfactory. The same criticisms apply to Pl. 1, fig. 1, of the present paper. The first requirement, then, of a good silver preparation is to show individual neurites arranged in parallel in bundles, with some indication of the structure of the sheath which supports them. The technique for doing this must be as reliable as possible and not subject to variation caused by uncontrollable factors. Rather than describe in detail all the several hundred separate experiments which have been done on fixation and silver impregnation, it is proposed first to give an outline of the technique which has proved successful, and then to provide in discussion form comments on its application.



Text-fig. 1. Histogram showing the distribution of neurite diameters measured from electronmicrographs of fifty intramuscular autonomic nerve bundles from rabbit small intestine.

A technique for the silver impregnation of autonomic nervous tissue in muscle coat preparations or frozen sections.

(1) Fix the intestine stretched lightly on a frame, or by filling the lumen with fixative and tying both ends while it is in a state of moderate distension, with 10 % formalin-sucrose prepared as follows:

Heat in a reflux condenser: trioxymethylene, 40 g.; sodium sulphite (anhydrous), 0·1 g.; brom thymol blue, 5·0 mg.; distilled water, 1000·0 ml. Near the boiling point the trioxymethylene goes into solution and the strong blue colour turns to the blue-green shade typical of pH 7·0–7·4. Prolonged boiling will turn the solution acid. Cool quickly in running tap water. Add 0·1 M sucrose. During the first half hour of fixation the formalin turns yellow-green or yellow, depending on the relative volumes of tissue and fixative. It should be kept neutral by the addition of very small amounts of sodium sulphite powder. Maintain the blue-green colour during 48 hr. and then allow the fixative slowly to turn acid. Continue fixation for not less than 7 days.

(2) Rinse the material in distilled water very briefly.

(*a*) Scrape off the mucosa from rabbit intestine, and proceed to stage 3.

(*b*) Scrape off the mucosa of cat intestine, remove the submucosa and then the circular muscle coat, and proceed to stage 3.

(*c*) Or cut frozen sections at 15–50 μ and store them in the fixative.

(3) Mount the material from a large dish of distilled water on slides coated with 5 % gelatine for thick pieces (2*a* or 2*b*), or 1–2 % gelatine for thin frozen sections (2*c*). Drain the preparation and cover with several strips of hard, smooth filter-paper soaked in water. Add a second slide to form a sandwich, and tie by winding a length of thick thread around both slides. Transfer to: absolute ethanol, 9 vol.; 40 % formaldehyde, 1 vol. A commercial solution of formaldehyde neutralized with chalk is suitable. Leave 1 hr. or longer.

(4) Remove the thread, top slide and filter-paper, and transfer to absolute ethanol for 5 mins. Clear in xylene for 5 min. or longer.

(5) Return the preparation to absolute ethanol, and pass it through an alcohol series made by diluting the alcohol with 10 % formalin made from a solution of 40 % formaldehyde stored over chalk.

(6) Drain the slide and wipe off the excess formalin to within the margins of the preparation. Place in the following silver solution for 10 min. or longer, pre-heated to 37° C.: 10 % silver nitrate, 1 vol.; 5 % cupric nitrate, 2 vol.

(7) Transfer the slides individually through three washing solutions and made up as follows: 2:4-dinitrophenylhydrazine saturated in distilled water, 1 vol.; 5 % glacial acetic acid, 1 vol. Two minutes in each dish with occasional agitation is a suitable time for washing.

(8) Transfer the drained slide to one of the following ammoniacal silver solutions in a small rectangular glass or plastic vessel:

(*a*) *Silver carbonate.* Dissolve 10 g. of $AgNO_3$ in 40 ml. of distilled water. Dissolve 8·42 g. of Na_2CO_3, 10 H_2O in 30 ml. of distilled water. Add the carbonate solution to the silver in small amounts from a pipette. The last few drops should be sufficient to complete precipitation. Wash the precipitate several times with a large volume of distilled water and, with the precipitate contained in a minimum volume of water, add strong ammonium hydroxide until the solution is almost clear. Dilute to 150 ml. with distilled water and filter.

For use take 6 ml. of the freshly filtered solution and add 3–10 drops of strong ammonium hydroxide (20 drops = 1 ml.).

(*b*) *Silver diammine solution.* This is made by adding strong ammonium hydroxide to 10 % silver nitrate until the precipitate is almost dissolved. Take 6 ml. of the filtered stock solution and add 3–10 drops of strong ammonium hydroxide.

(c) *Silver diammine solution* to which is added 1 vol. of 5% sodium carbonate per 100 vol. Take 6 ml. and add 3–10 drops of strong ammonium hydroxide.

As soon as the slide is immersed in one of the above silver solutions, add 2 drops of 5% formalin (20 drops = 1 ml.) made from trioxymethylene with a trace of brom thymol blue and adjusted approximately to pH 6·5 by the addition of sulphite. It is important to add the formalin while agitating the silver solution to avoid local precipitation. The impregnation develops best at a temperature not below 20° C. The staining should be checked under a microscope.

(9) Wash the preparation in a large volume of 5–10% sodium sulphite, with several changes for 5 min. Wash in running tap water.

(10) Dehydrate through an alcohol series, clear in xylene and mount in Canada balsam or some synthetic medium.

The above technique will now be discussed in relation to the many experiments which led to its formulation.

Fixation

The outstanding obstacle to closer correlation between the results obtained by silver impregnation and by electronmicroscopy is undoubtedly the relatively poor quality of the formalin fixation. Once one becomes accustomed to the criteria established for good fixation in electronmicroscopy it is difficult to tolerate the inferior fixation image of the standard methods used in light microscopy. None of the alternatives to formalin, such as fixation with chloral hydrate, alcohol and ammonia, or picro-formol solutions, is suitable. Osmic or chromium fixatives are, or course, quite incompatible with the type of silver impregnation used. Commercial formalin is a variable product often containing as much as 10–15% methanol added to inhibit polymerization. A large series of different formalin solutions was investigated, including many made up in phosphate, acetate, boric, veronal, tris and collidine buffers. Also tested were the different classical methods of neutralizing formalin such as calcium, magnesium or lithium carbonates or pyridine. In general the results were unsatisfactory either because the fine AGP bundles were too distorted, or because the impregnation failed altogether. If the fixative had been kept at pH 7·5–8·0 by a buffer, the impregnation of the AGP was often inhibited, and a quite different result favouring dark staining of Type 1 neurones in Auerbach's plexus was obtained. The addition of buffer, particularly boric-borate, caused distortion of the AGP with coarse beading of its neurites.

The formalin-sucrose solution made from trioxymethylene remains very stable at room temperature. During fixation the pH is easily re-adjusted and it seems to be important to allow the solution finally to turn acid in order to get optimal uptake of silver in the AGP components. It is recommended that sufficient brom thymol blue indicator to give a strong blue-green colour be used to follow the fluctuations of pH during the initial stages of fixation. Even though the tissue may be stained with the indicator during fixation, the silver impregnation is apparently unaffected. Adjusting the tonicity of this formalin with sucrose considerably improved the preservation. It is interesting to note that the quality of fixation deteriorates with the addition of 1–2% methanol. As for the duration of fixation there are two alternatives. Either the material must be left for several months in formalin, after which the impregnation can be undertaken without mounting and extraction with alcohol or xylene, or shorter fixation must be combined with extraction. Presumably it is necessary to allow sufficient time for formalin in the first case to dissolve out certain reducing substances, or in some way render them non-argyrophil. Alternatively with shorter fixation periods these substances can be removed by alcohol and xylene. The only drawback to the latter procedure is the shrinkage distortion produced by the alcohol. It is interesting to note that material could be stored if necessary in xylene for several months without detriment to the reaction with silver. Even exposure to pyridine at 60° C. for 7 days leaves the material unharmed. Thus there is no evidence that any particularly subtle factors of fixation are important apart from pH control and careful attention to the quality of formalin used. It is not insisted that formalin made from trioxymethylene is superior to all commercial solutions, but merely that this method of preparation is a safe one to use until a commercial solution is found which regularly gives identical results.

Mounting the material

The conventional technique for mounting frozen sections on gelatinized slides in formalin vapour is quite unsuitable for this technique probably because the material is made too acid. The method described is a very reliable one and seems to involve less distortion of the material than would result if sections or pieces of material were carried through the alcohols unattached to slides. It is important not to tie the slides too tightly or an imprint of the filter-paper will be embossed on the sections. A mounted preparation of rabbit intestine should be firmly enough attached at the xylene stage to allow the submucosa to be stripped, using watchmaker's forceps, with the preparation immersed in a dish of xylene under a dissecting microscope.

Material extracted after mounting must be soaked again in formalin before silver impregnation. It was consistently found that the combined circular and longitudinal muscle coats from the rabbit, in which the nerve plexuses remain sandwiched between protective layers of muscle, showed more extensive impregnation of the AGP following washing in water, than cat material in which the nerve plexuses had been exposed by stripping the circular muscle before impregnation. Frozen sections did not give any impregnation of the AGP at all if they had been mounted, extracted and then passed through an alcohol series to water. It was decided, therefore, to test whether removal of formalin was the factor causing inhibition. After passing the material from xylene through alcohol solutions containing formalin and finally into 10 % formalin, these discrepancies were removed. Thus a probable explanation could be given as to why numerous experiments involving treatment of the fixed material with buffer solutions before silvering had produced inhibition of AGP staining, especially with cat preparations. Various attempts to 'mordant' the material before impregnation by treatment with alcohol-ammonia, or salts of copper, cobalt and uranium had also failed, partly no doubt for the same reason.

The type of formalin used immediately before silvering turned out to be quite important. Thus alkaline formalin produced strong staining and mirror formation in the gelatine coating of the slides, as well as inhibiting AGP staining. Formalin at pH 5–6 was the most suitable, and here it was interesting to compare a solution made from trioxymethylene at this pH range, with a commercial formalin stored over chalk at approximately the same pH when diluted. Both solutions gave satisfactory impregnation, but the reaction was more intense and, if anything, more complete with the formalin containing calcium formate. Evidently sufficient calcium goes into solution in acid formalin to cause an appreciable increase in the uptake of silver.

Initial silver impregnation

The silver technique recommended above is, of course, based upon the well-known Bielschowsky–Gros method (see Garven & Gairns, 1952), which in brief consists of an initial impregnation in a strong solution of silver nitrate, washing out excess silver with formalin diluted in tap water and then developing the image with silver diammine. From the work of Holmes (1943), Samuel (1953) and Peters (1955) there is abundant evidence to show that the pH of weak silver solutions profoundly influences the nature and usefulness of silver impregnations, particularly of nervous tissue in the form of paraffin sections. The behaviour of strong silver nitrate solutions has not been investigated with the same care. Evidence was obtained in an early part of this work that weak silver solutions (e.g. 1/20,000 $AgNO_3$) in acetic-acetate buffer at pH 5·0, followed by the conventional Bielschowsky–Gros technique, gave an impregnation of Schwann cell cytoplasm, interstitial cells and nuclei, with the neurites unspecifically stained. On increasing the acidity the silver became specific for nuclei and then it failed altogether. On the other hand, if 10 or 20 % silver nitrate was used, without adjusting the pH the tendency was nearly always towards coarse neurite and perikaryon staining with an incomplete impregnation of the finer plexuses. Since no manipulation of the pH of weak silver solutions gave satisfactory results, either in the acid or the alkaline range, it was decided to work with more concentrated silver, adjusting the pH by the addition of nitrates of various other metals. It was necessary to work with a pH meter having a bridge containing saturated KNO_3 between the glass and the calomel electrodes as described by Datta & Grzybowski (1959).

The pH of 10 % silver nitrate is about 4·3. By dilution of this silver solution with different proportions of a 5 % solution of one of some seventeen different metallic nitrates, a range of pH values was obtained. It was soon evident that those nitrates which lowered the pH of silver nitrate also caused the impregnation to shift from the coarse neurites to the finer ones, and eventually exclusively to the Schwann sheath, to the nuclei and then to fail altogether. Cupric nitrate was by far the most successful in modifying the impregnation, the pH of the standard solution quoted above being 3·4. It was interesting to note that those nitrates which raised the pH above 4·3 made the impregnation much coarser. Some completely abolished all trace of the AGP. Attempts to reveal the AGP in the form of a completely specific staining of its neurites, leaving all other components unstained, were liable at best to reveal only a few of the thicker neurites in an AGP bundle, and to miss much of the detail of the relationships of the AGP to the surrounding tissues.

It cannot be concluded without careful investigation that the effect of adding cupric nitrate to the silver solution is entirely a matter of changing the pH. All that can be said is that the result has been obtained largely by empirical experimentation, but it works reliably without significant deviation. We have used a temperature of 37° C. merely to keep the degree of impregnation constant for a given time interval. The impregnation may have to be extended in some cases to as much as 1 hr. More prolonged exposure results in so much uptake of silver that the muscle tissue is too dark to reveal the AGP clearly. The silver solution needs renewal as soon as it becomes discoloured by the addition of traces of formalin.

Completion of the impregnation

The suceeding stages of the Bielschowsky–Gros technique, involving repeated washing of the silver-soaked material in formalin baths diluted with tap water, are hardly designed to allow one to control the final impregnation with any accuracy. It is well known that some histologists extol the merits of the tap water in their particular region as contributing to the success of their preparations. In order to make the technique more controllable, particularly with regard to the reduction phase in ammoniacal silver, it was decided to wash out the excess silver with a reducing solution which had a very slow reaction with ammoniacal silver. Then, if a small measured amount of formalin was added after placing the material in ammoniacal silver, the reduction could be controlled more easily. A saturated solution of 2:4-dinitrophenylhydrazine, or a similar solution diluted with an equal volume of 5% acetic acid, will wash out excess silver quite effectively without damaging the initial impregnation. Incomplete washing, of course, leads to precipitation.

There is little to choose between the three ammoniacal silver solutions recommended above, except as regards contrast and freedom from artefact. The silver carbonate solution gives a somewhat low contrast because the muscle is stained yellowish brown. It is to be recommended for initial attempts with the technique because the reduction proceeds slowly and smoothly. The silver diammine solution containing sodium carbonate is also easily controlled and gives greater contrast. The simple diammine solution is more difficult to control because the addition of formalin sometimes results in local precipitation. If these solutions are made up carefully with no excess of ammonium hydroxide, filtered before use, and measured accurately for each repetition of the technique, very constant results should be obtained. As regards the amount of excess ammonium hydroxide there can be no strict control as so much depends on the strength of the stock solution, but the important point to bear in mind is not the absolute amount of excess ammonium hydroxide in the silver bath but the relative proportions of ammonia and formalin. A preliminary test of the stability of an ammoniacal silver solution to formalin in the presence of excess ammonia should be made. Take 6 ml. and add 1 drop of the washing solution in section 7, and 2 drops of the 5% formalin. If the correct amount of excess ammonium hydroxide has been added the solution will remain clear during 5 min. and then begin to turn light grey. A silver carbonate solution containing a small excess of sodium carbonate, inadvertently added during preparation, will be found by the above test to need less ammonium hydroxide for suitable stability. One can get a successful impregnation from a solution which contains as much as 10 times the usual amount of ammonium hydroxide provided a comparable excess of formalin is added. Thus we have the situation more closely under control with the present technique than is possible with the classical Bielschowsky–Gros method, where variable amounts of formalin are introduced into the silver bath, depending on the size of the pieces and how much they have been drained beforehand.

The impregnation develops in the course of about 3 min., during which the solution remains clear. A prolonged staining time of 30 min. or more may be obtained by adding only a single drop of formalin to 6 ml. of silver solution. Generally speaking, if frozen sections contain strongly argyrophil muscle and epithelium, a slow impregnation in ammoniacal silver is better than a fast one. If the impregnation is, however, too slow in developing, more formalin may be added during staining, but eventually the silver solution will become turbid with the risk of depositing silver precipitate on the preparation. Slow staining will allow the slide to be taken from the ammoniacal silver, mounted with a coverslip, and observed even under oil immersion if necessary, while the silver solution continues its action. This is sometimes a great convenience in judging the exact stage of impregnation in frozen sections.

The usual rinsing baths and treatment with sodium thiosulphate have been replaced by sodium sulphite because this reagent checks further reduction of the silver more quickly, and is quite effective in removing all unreduced silver. It will be noted that addition of a large excess of formalin to the ammoniacal silver to speed the reaction, as described above, sometimes results in a preparation which continues to darken appreciably during treatment with sulphite. It is necessary in such cases to increase the sulphite concentration. During dehydration the circular muscle coat of rabbit intestine can be stripped if required at the 50 % stage.

The above technique is applicable, of course, to unmounted material or sections but, if these are to be extracted before impregnation, there will be gross shrinkage followed by a compensatory swelling in the ammoniacal silver solution. The great convenience, however, of having frozen sections at 15–50 μ mounted before manipulation in the various staining solutions is an attractive feature of the technique. Serial frozen sections can be handed with ease, and the size of the slide and coverslip is really the only limitation to the area of the preparation which can be successfully impregnated. Assuming that analytical grade reagents will be used, and reasonable care taken in the preparation of the distilled water, there is not much room for failure with the technique as presented. Considerable latitude exists, of course, for varying it and one can do this with much closer control than was possible with the original method. The technique has been equally successful using reagents purchased in Great Britain and in the U.S.A.

PART II

Comparison of the electronmicroscope and silver-stained images

If we now return to the tertiary bundles of Auerbach's plexus and their extensions in the muscle coats, it will be seen from Pl. 2, fig. 2, that the tertiary bundle stained with silver consists of very thin neurites coursing longitudinally, deviating a little to pass by a Schwann cell nucleus, or to enter a smaller branch on the way to the muscle. Branching of these neurites on the surface of the bundle can sometimes be clearly seen, Referring to Pl. 2, fig. 8, from an electronmicrograph of a sectioned tertiary bundle we see that the silver-stained image now bears comparison in that the number of thick neurites reaching the surface of the bundle either on the left- or the right-hand side is approximately the same as the number we can distinguish in the silver-stained bundle. The total number of neurites, of course, will never be seen in such a bundle because some are too small to be resolved by the light microscope and only those on the upper surface of a bundle can be brought sharply into focus. Pl. 2, fig. 3, shows a silver-stained bundle of much smaller diameter coursing between the muscle fibres of the longitudinal coat. It is possible to identify about five separate neurites in this bundle which, on changing the focus, preserve their identity throughout and do not fuse to form a network. This illustration is the counterpart of the electronmicrographs (Pl. 3, figs. 5–7) where again there are neurites too thin to be resolved in silver preparations.

One of the advantages of the method of silver impregnation is that it can be applied to the region of the mesentery and the points of entrance and exit of blood vessels and extrinsic nerves. Here the thickness of tissue to be stained is considerable and the area is often packed with adipose tissue. Pl. 3, figs. 9, 10, illustrate the extraordinary wealth and complexity of the nerve supply associated with the arteries and veins. There is an intimate peri-vascular plexus of thin bundles in the adventitia, which accompanies the vessels throughout their length, receiving branches from thin bundles coursing alongside the vessels, and from the bundles of Auerbach's plexus nearby. Also running parallel with the vessels are thick nerve bundles which join Auerbach's plexus at various points close to the area of attachment of the mesentery. A suitable degree of staining allows detailed examination of the peri-vascular plexuses as shown in Pl. 3, figs. 11, 12. Once again the finest nerve networks are shown to consist of bundles of parallel neurites with a Schwann sheath. The continuity of this fine network with a thick bundle of neurites running across the blood vessel is illustrated in Pl. 3, fig. 11.

Another aspect of the structure of the nervous tissue in the intestinal wall, which has been examined by the two techniques, concerns the problem of synaptic endings in the ganglia. These have been identified in electronmicrographs (see Richardson, 1958) as being present on the surface of the ganglion cells and on their neurites close to the perikaryon. They are very small structures which have never been shown convincingly in intestinal ganglia by silver impregnation. Although the silver technique here described is more specific for neurites of medium and small diameters than other methods, it falls short in demonstrating these neurites completely at their terminations. The nearest we have been able to get is represented in Pl. 4, fig. 14, where a leash of fine neurites is coiled around the thick process of a Type II neurone, and a few ring-shaped bodies, reminiscent of larger synaptic endings elsewhere, are somewhat faintly resolved.

Impregnations carried out at a pH below the optimum for neurite staining, e.g. by using a weak silver solution buffered with acetic-acetate for the initial impregnation, give prominence to the interstitial cells and to connective tissue cells in general. These have been examined in three situations: (1) in the serosa (see Pl. 5, fig. 17); (2) within the muscular tissue (see Pl. 5, fig. 15); and (3) in the interval between the longitudinal and circular muscle coats (see Pl. 5, fig. 16). Since we are dealing with total cells and not sections of cells in stripped preparations, there is an opportunity to examine the morphology of these cells almost as though they were in tissue culture. The over-all impression is that these cells all belong to a population of connective tissue cells distributed at random. They are not arranged in any distinct pattern related to the distribution of the AGP, though of course they are in rather intimate relationship with the AGP because both these components happen to lie in very restricted spaces in the tissues. Within the muscle they are not numerous enough to form a connected network, and in electronmicrographs no intimate membrane to membrane relationship with neurites or with muscle cells has been seen. The cells vary a little in their general contours and in the nature of their processes. Thus in the serosa the processes are irregular and branch repeatedly into extensions of unequal width. They do not taper smoothly to a fine termination. A few cells like these can be seen in the interval between the muscle coats, but the majority of the cells in this situation show smoother processes which are extremely elongated. Here the resemblance to neurones is more evident, but at the electronmicroscopic level the processes are found to be very irregular and not at all comparable with neurites. As these connective tissue cells have no basement membrane it is unlikely that they are related to Schwann cells.

PART III

Discussion

The controversies concerning the fine structure of the autonomic innervation of tissues and organs have been pursued in the past partly with the assumption that the process of fixation immobilizes the components of a tissue in some sort of standard state, which can be assumed to be reasonably constant from one investigator to another, and that variations in the optical image are due to different elements of this standard fixation state receiving emphasis from one stain or another. Thus when one school claimed that autonomic nerves were much more abundant and more extensively distributed in the body, forming ultimately what was described as a terminal reticulum, it was commonly supposed that argyrophil connective tissue fibres were being confused with neurites. Since the introduction of electronmicroscopy we now have a better idea as to what good fixation entails and indeed how difficult it is to achieve it with most of the fixatives used in light microscopy. We have seen that the composition of the initial fixative must be carefully controlled and that a final pH on the acid side favours the impregnation of the finest neurites, while suppressing the heavy impregnation of perikaryons and thick neurites so typical of the conventional Bielschowsky–Gros preparations. There need be no confusion between connective tissue fibres and neurites, not only because these components are so distinct in electronmicrographs, but also because the technique here described does not stain collagen more than a faint yellow, unless it has been grossly torn and exposed to oxidation. Ragged strands of submucosa left, for instance, on the surface of a mounted preparation of intestine, occasionally show a darker impregnation.

If we take some of the illustrations of Stöhr (1957) in his extensive monograph on the autonomic nervous system in the light of the present findings, it does not seem unreasonable to say that fig. 101, 102 and 103 represent undoubted nervous tissue, not connective tissue, but in the form of rather badly fixed AGP revealed by specific staining of distorted neurites. The bundles appear grossly swollen and in some cases disrupted, so that the orderly arrangement of the neurites has become distorted into networks. Turning to fig. 111, we see the result of bad fixation combined with an impregnation too far on the acid side, in which the infoldings of the Schwann cell membranes (mesaxons) are emphasized at the expense of the neurites. These are not interstitial cells as labelled in this figure, but badly fixed Schwann cells showing not neurofibrils, but incompletely stained neurites. When we turn the page to fig. 114, further confusion arises because a badly fixed part of the AGP has been illustrated from an incompletely impregnated preparation! The same criticisms apply to the work of Boeke, illustrated, for instance, in fig. 133. Here the AGP of a sweat gland shows Schwann membrane staining rather than the typical system of parallel neurites.

In adopting a critical attitude to much of the work done by silver impregnation it would be a mistake to lose sight of the fact that the total distribution of autonomic innervation is vast as Stöhr's monograph undoubtedly shows. Its components are built on an exceedingly fine scale for which crude techniques such as fixation in chloral hydrate and alcohol, paraffin embedding and impregnation with weak silver solutions, are quite inadequate and misleading. Indeed observations with the electronmicroscope seem to indicate that part of the final autonomic pathway consists of neurites below the limits of resolution by light microscopy no matter how precisely they may have been stained.

Another source of confusion arose in the pre-electronmicroscope era from the assumption that intravitam methylene-blue staining could be relied upon to delineate neurites specifically in all situations, and that networks stained with this dye, which did not appear to consist of bundles of neurites, were in fact composed of cells similar to the interstitial cells originally described from Golgi-stained preparations. It has been shown that these cells exist independently from the AGP in the small intestine, and that the conception that they provide a link between the AGP neurites and the effector cells can hardly be sustained. If these cells cannot be separated in a particular preparation from the AGP then they are simply Schwann cells stained in such a way that the neurites they support are invisible. Referring again to Stöhr (1957) we find in fig. 106 a well-fixed preparation of the wall of a uterine artery showing a nucleated network stained with Ehrlich's haematoxylin. This stain does not emphasize neurites, with the result that the network appears to consist simply of cells with branching and interlocking processes. It is wrong to label these as interstitial cells when it is obvious that a suitable silver impregnation would show the network to contain bundles of neurites in continuity with the larger autonomic bundles supplying the artery, as described in an earlier part of the present work. Indeed the first essential, if a network is revealed in these situations, is to adjust the fixation and staining so that neurites can be traced into the network in conformity with what we know of the ultrastructure of this tissue. This has been done recently by Hillarp (1959) using methylene-blue staining. The present author is in complete agreement with his findings and the discussion he provides needs no further elaboration here.

A discovery which may be relevant to the controversy concerning the identity of interstitial cells has been reported recently by Benitez, Murray & Chargaff (1959). These authors have shown that microsomes from rat liver or kidney, added to cultures of adult rat subcutaneous fibroblasts, induce a striking change in the morphology of the fibroblasts. They develop extremely long processes and take on the general appearance of cultures of nervous tissue. Similar effects could be obtained by the fractionation of microsomes or by adding purified RNA. At least we know now that fibroblasts can show under special experimental conditions a morphology which is very similar to the characteristic cells found in the interval between the muscle coats of the intestine. Mechanical factors, such as the type of surface with which connective tissue cells in the gut wall make contact, may also play a part in determining their shape, particularly the contours of their processes. The influence of the type of medium and the surface on which cells are growing has long been known to be important in determining the morphology of cells *in vitro*. In the serosa the substratum with which fibroblasts are associated is a mesh work of collagen bundles, in the muscle interspaces a quite different stroma consisting of a reticular fibre meshwork occurs. Thus in Pl. 5, fig. 16, the majority of the cells in question lie in a fairly wide interval where possibly they are free to extend their processes for long distances. A few cells in this population, however, are in contact with the surface of the muscle and this may influence them to spread their processes into broad, sheet-like extensions similar to those of the serosal connective tissue cells which interweave amongst the collagen bundles. The opportunity to view such a population of sharply stained connective tissue cells *in toto* without sectioning, and *in situ* in their normal position in the tissue, comes rarely, and we do not have sufficient experience with the full range of morphological variation such cells may undergo.

SUMMARY

1. The problem of equating the structural appearances of fine autonomic neurite bundles in silver-impregnated preparations of small intestine viewed with the light microscope, with similar structures in electronmicrographs of permanganate-fixed material, has been investigated.

2. Numerous experiments on fixation and methods of silver impregnation have led to the formulation of a technique which is reliable for whole mount preparations and frozen sections in giving emphasis to individual neurites in bundles as small as 2–3 μ in diameter.

3. This technique provides a light microscopical image of autonomic nerves which compares favourably with what we know of their ultrastructure, but the inferior quality of formalin fixation, combined with lack of sufficient resolution, is likely always to hinder complete correlation.

4. A variety of appearances, due mainly to Schwann sheath staining rather than neurite staining, can be obtained by using commercial formalin without adjusting the tonicity and pH and by varying the pH of the initial silver impregnation. Variations such as these form the basis of many of the conflicting accounts of the fine structure of autonomic nerves of previous authors.

5. At a suitable pH below that for optimal neurite staining, emphasis is given to connective tissue cells including the interstitial cells. Differences in the morphology of these cells are described for several regions in the gut wall. Their arrangement and relationship with the nervous tissue does not support the contention that interstitial cells form a link between neurites and smooth muscle fibres.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Whole mount of rabbit longitudinal muscle coat impregnated with 1/20,000 $AgNO_3$ in m/10 acetic-acetate, pH 5·5, showing secondary and tertiary bundles of Auerbach's plexus. The clear distinction between dark-staining nerve bundles and pale-staining interstitial cells (fibroblasts) is evident, but the silver emphasizes the Schwann sheaths rather than the neurites. At high magnification the fine structure of the AGP in this preparation could not be resolved.

Plate 2

All figures in this plate are from rabbit small intestine.

Fig. 2. A tertiary bundle from a whole mount of Auerbach's plexus, standard silver technique. About 15–17 neurites can be resolved on the surface of this bundle which is about 7μ in diameter. Compare with fig. 8 which is an electronmicrograph of a sectioned bundle about $5{\cdot}5\mu$ in greater diameter. About 10–12 neurites reach the surface on the right or left sides, which corresponds to what would be seen in a whole mount.

Fig. 3. A thin AGP bundle within the longitudinal muscle coat of a whole mount, standard silver technique. At different levels of focus five to six individual, longitudinally orientated neurites can be resolved. Compare with fig. 7 which is an electronmicrograph of a similar bundle in transverse section.

Fig. 4. An intramuscular AGP bundle showing one thick, two medium-sized and four very thin neurites surrounded by a Schwann sheath with the exception of the neurite which lies between the sheath and the basement membrane. The edges of smooth muscle fibres appear above and below the nerve.

Fig. 5. An intramuscular AGP bundle containing five neurites and a single irregular area of Schwann cytoplasm. The two neurite profiles below the bundle appear to lie outside the basement membrane.

Fig. 6. An intramuscular AGP bundle containing four neurites. Numerous profiles lie in the space between the smooth muscle fibres in this field. Some of them may be neurites but the permanganate fixation gives no specific criteria for their identification.

Fig. 7. An intramuscular AGP bundle containing 10 neurites and five separate areas of Schwann cytoplasm. Below the bundle is part of a process of an interstitial cell (see fig. 15).

Fig. 8. A tertiary bundle from Auerbach's plexus. Surrounding the bundle are processes of interstitial cells and, at the upper margin of the field, part of the nucleus of one of these cells is included. At the left lower corner is the edge of a smooth muscle fibre belonging to the longitudinal coat.

Plate 3

(All figures in this plate are from whole mounts of cat small intestine.

Fig. 9. An artery, a vein and part of a primary bundle of Auerbach's plexus near the point of attachment of the mesentery. An extrinsic nerve bundle on the right passes parallel to the artery to join Auerbach's plexus elsewhere. Closer to the artery are longitudinal bundles which form a peri-arterial plexus receiving contributions from the primary Auerbach bundle on the left.

Fig. 10. Vascular innervation near the attachment of the mesentery. A single plane of focus gives a poor indication of the amount and complexity of the nerve networks entering and leaving the gut wall.

Fig. 11. Part of the adventitia of an artery similar to that in fig. 10. A thick bundle of neurites crosses the artery transversely to join the parallel bundles. From this bundle emerge very thin bundles of neurites which form a typical AGP. If the focus is changed, this plexus can be seen to be continuous over most of the adventitia. Such a network stained with methylene blue is often wrongly described as formed of interstitial cells.

Fig. 12. Two areas of the fine-meshed arterial AGP at high magnification, seen against a background of elongated smooth muscle nuclei which are out of focus. The separate identity of the individual neurites can be traced for long distances in this network if the focus is changed. The lower area shows a typical Schwann cell nucleus which interrupts the parallel order of the neurites.

Plate 4

Both figures are from cat small intestine stained with the standard method.

Fig. 13. A small cluster of ganglion cells associated with a secondary bundle of Auerbach's plexus. The considerable number of fine neurites traversing the surfaces of the perikaryons makes the density of a larger ganglion too great for photomicrography. The cat intestine is too thick for satisfactory immersion fixation of the tertiary bundles which appear distorted in this preparation.

Fig. 14. Three ganglion cells from Auerbach's plexus. The largest shows a thick process passing to the lower margin of the field which has a leash of fine neurites wrapped around it. The arrows point to possible synaptic endings.

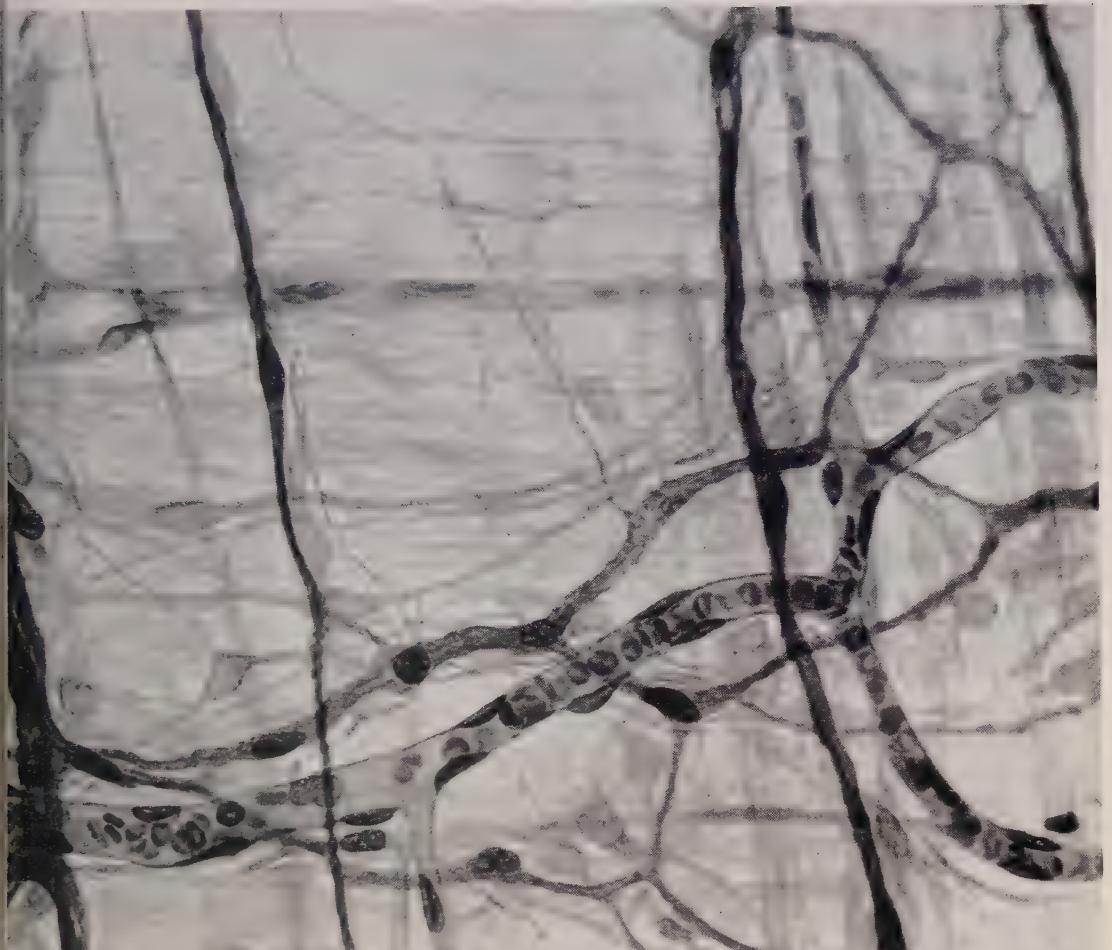
Plate 5

All figures from whole mounts of rabbit small intestine impregnated at a pH below the optimal for neurite staining.

Fig. 15. From the longitudinal muscle coat showing an interstitial cell associated with an AGP bundle. The nucleus of the interstitial cell is pale and surrounded by granular cytoplasm, which is extended into extremely elongated processes, which lie in the spaces between smooth muscle fibres.

Fig. 16. Showing an area of densely crowded interstitial cells in the interval between the circular and longitudinal muscle coats. The arrangement of their elongated processes does not suggest any significant relationship either with the tertiary nerve bundles or the smooth muscle fibres in the background.

Fig. 17. Cell types of the serosa seen against the background of interlacing collagen bundles. The granular cell at the upper right margin is a macrophage.



RICHARDSON. STRUCTURE OF AUTONOMIC NERVES IN THE SMALL INTESTINE

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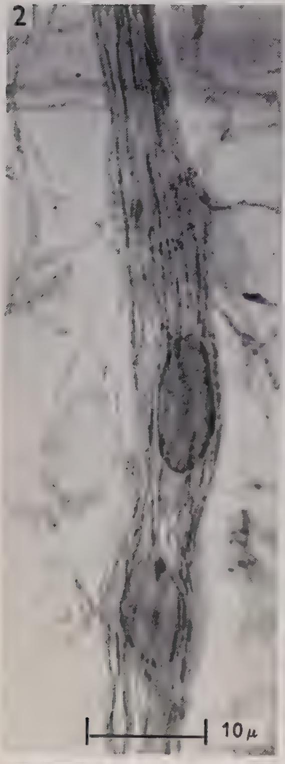

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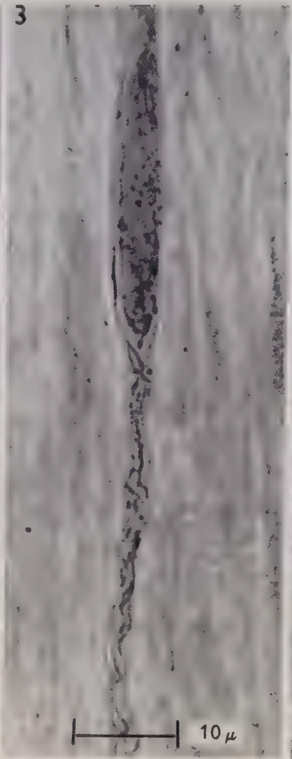

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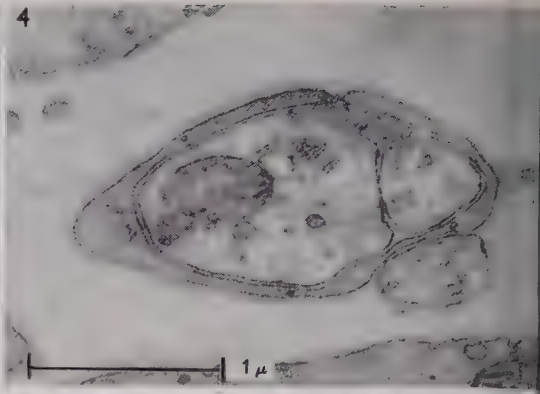

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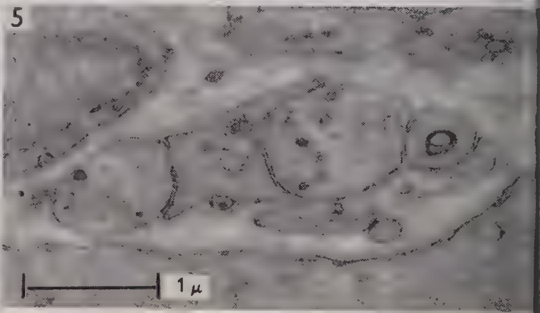

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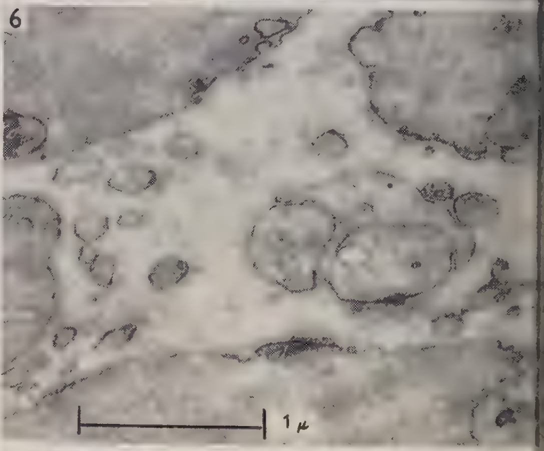

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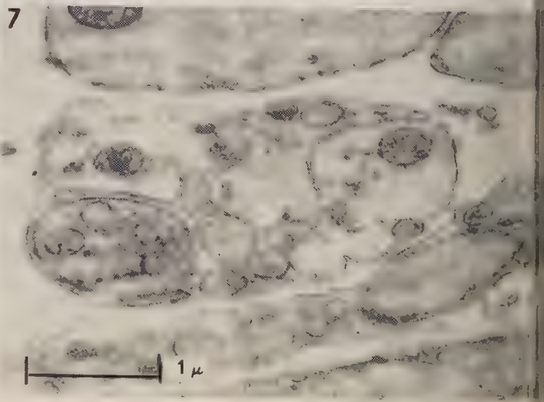

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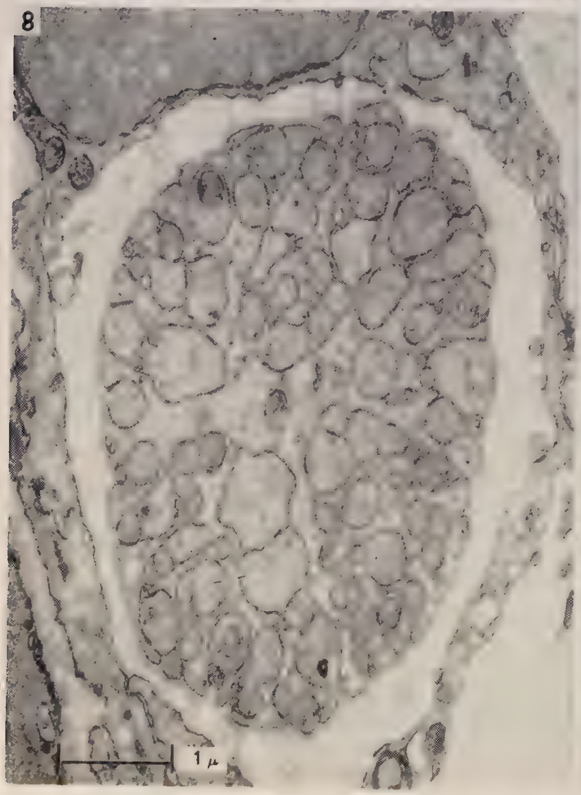
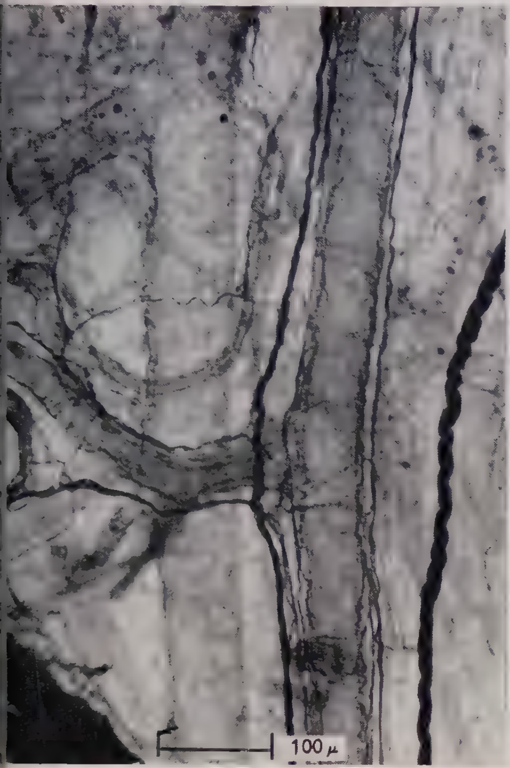


RICHARDSON. STRUCTURE OF AUTONOMIC NERVES IN THE SMALL INTESTINE



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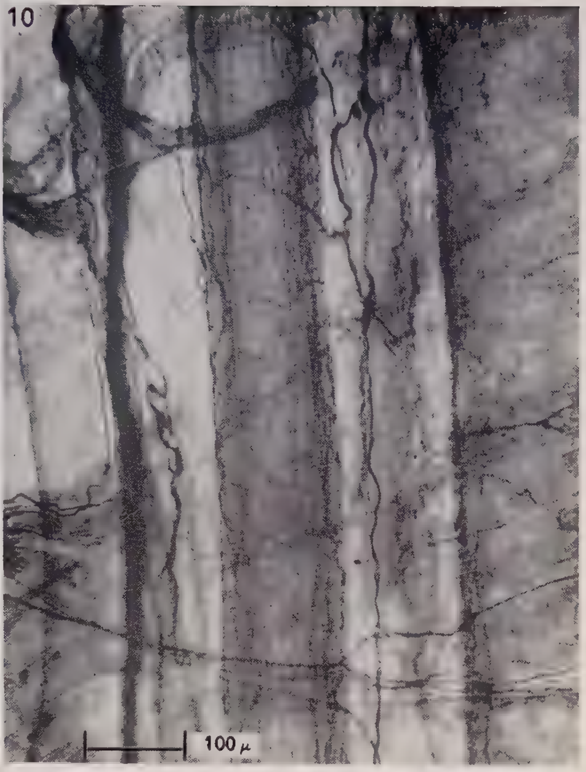

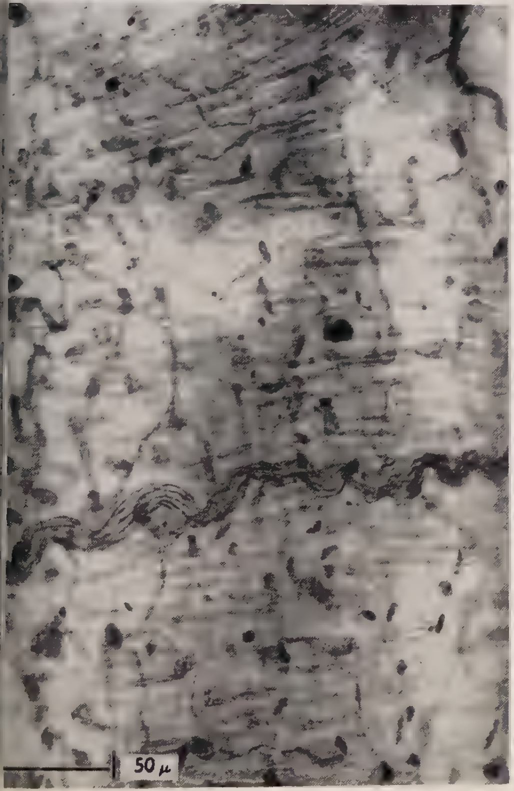

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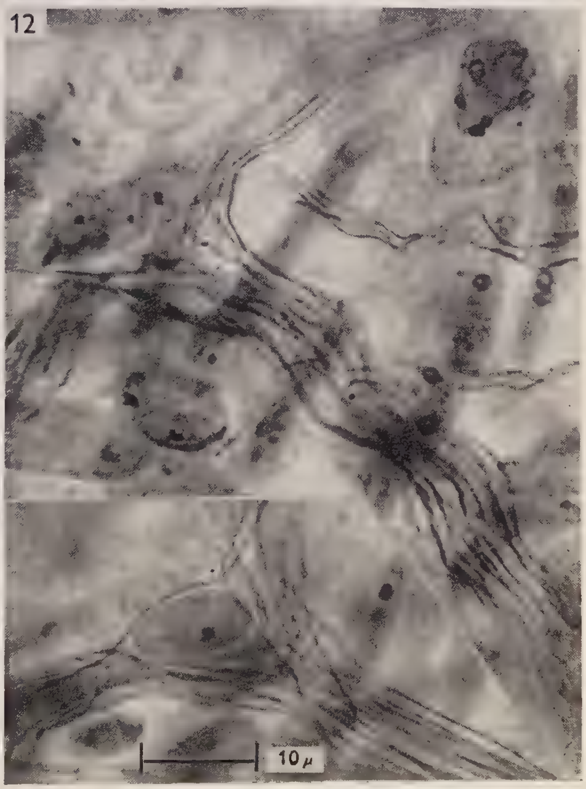

RICHARDSON. STRUCTURE OF AUTONOMIC NERVES IN THE SMALL INTESTINE

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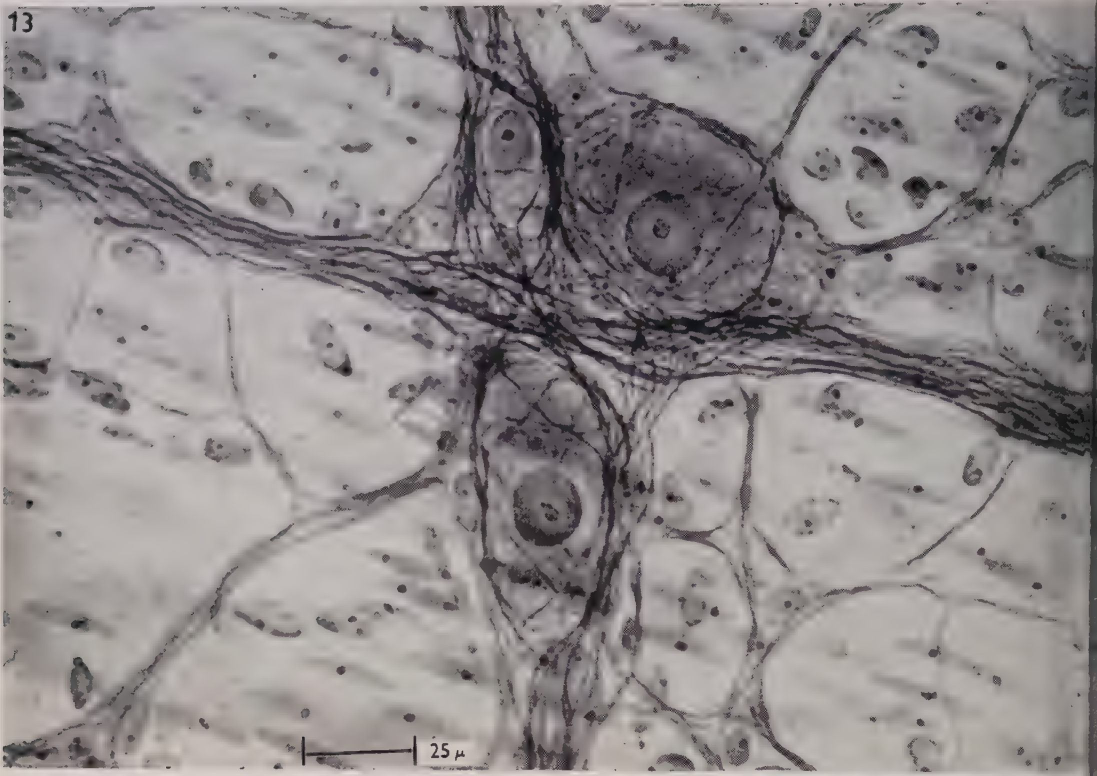

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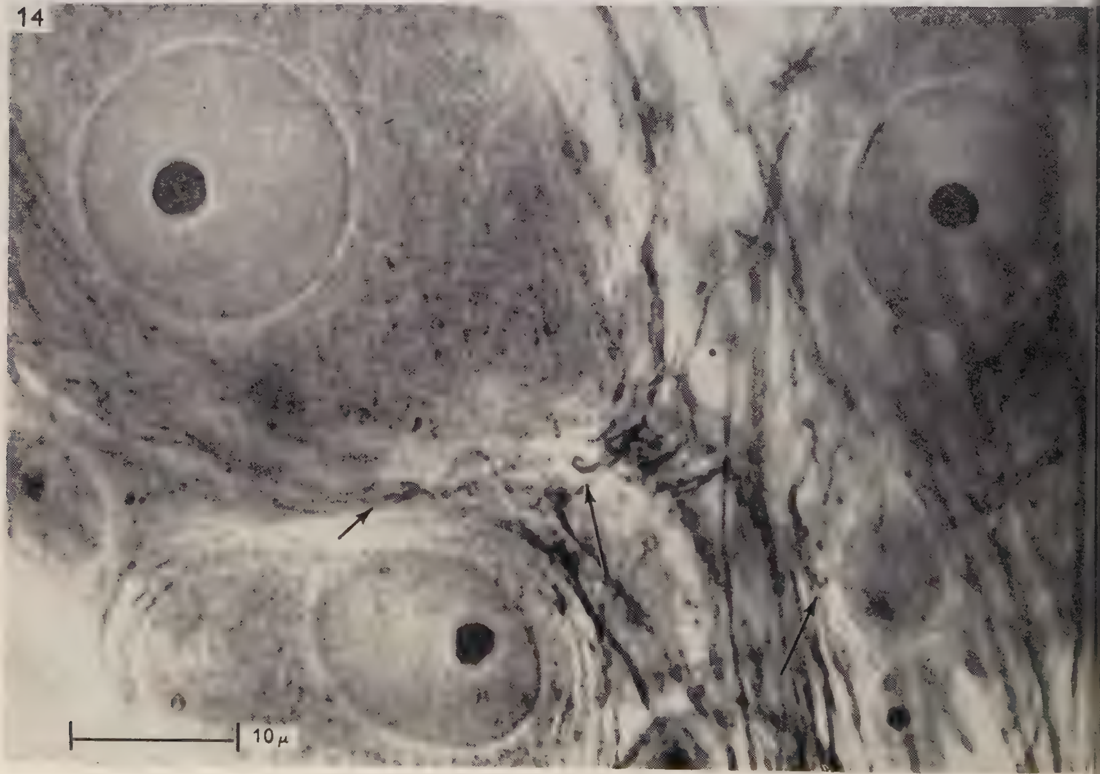
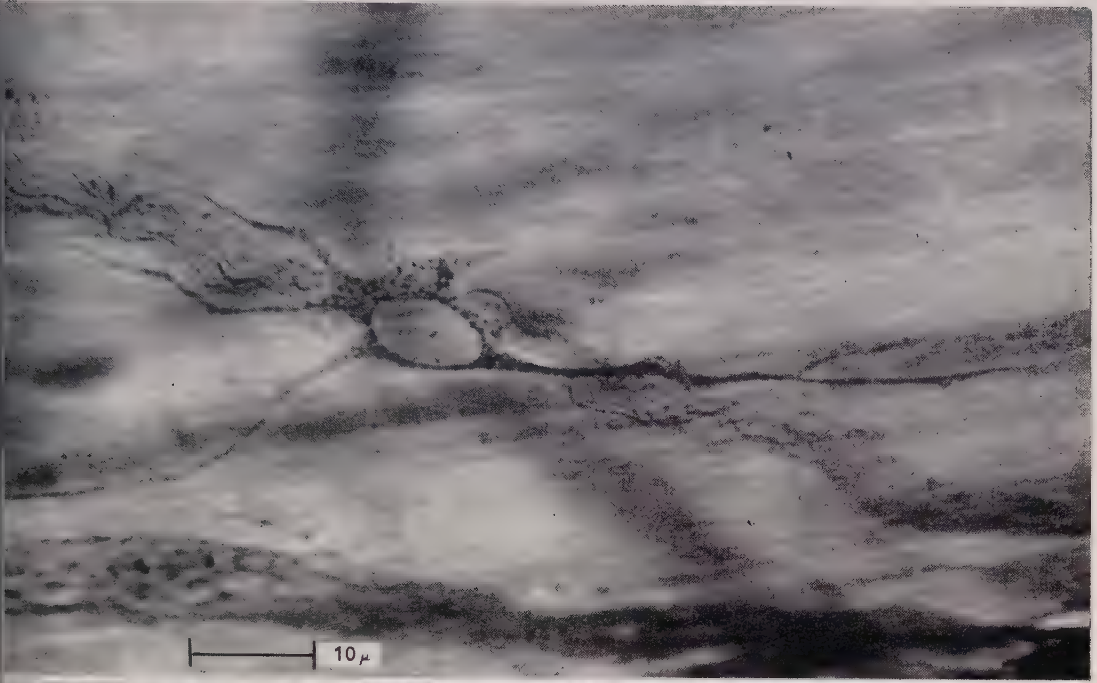
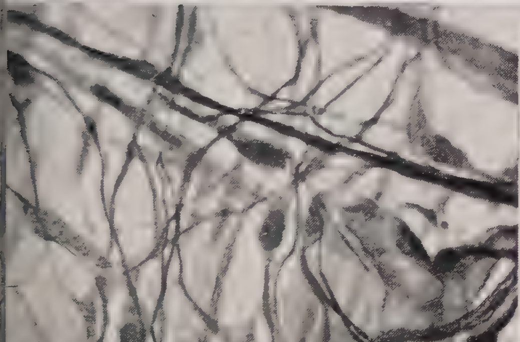


RICHARDSON. STRUCTURE OF AUTONOMIC NERVES IN THE SMALL INTESTINE





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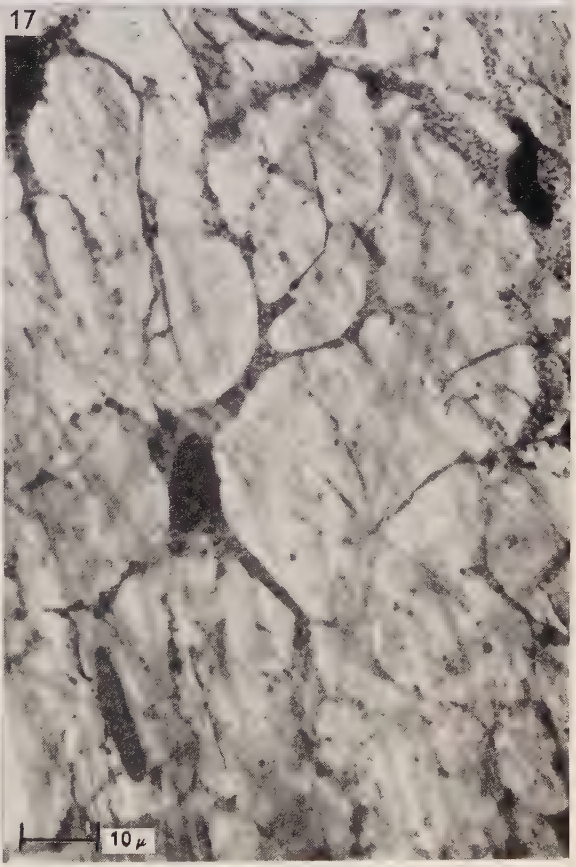

RICHARDSON. Structure of autonomic nerves in the small intestine

THE NUMBER AND DISTRIBUTION OF MUSCLE-SPINDLES IN CERTAIN MUSCLES OF THE CAT

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As a preliminary to quantitative studies of the proprioceptive equipment of various muscles in the cat, it became necessary to determine whether there was any correlation between the number of muscle-spindles contained in a given muscle and in its partner on the opposite side of the animal. Such a correlation might be expected as part of the expression of the bilateral symmetry of the animal. Although Morpurgo (1897) reported eight spindles in each sartorius muscle of the rat, Tower (1932), in a study of the interosseous muscles of the cat's forelimb, counted twenty-two spindles in the left Vth interosseus as compared with fourteen in the right, and concluded that there was little correspondence between the spindle content of identical muscles on opposite sides of the same individual. Apart from these observations the literature lacks any reference to paired counts of spindles (i.e. counts of the spindle content of a pair of identical muscles from the same animal). Those who have pursued such quantitative studies in the past (e.g. Gregor, 1904; Voss, 1937) have been primarily concerned with determining the spindle content of individual muscles and devising different methods of expressing 'spindle density', i.e. the proportion of the spindle content to the weight or volume of the muscle, or the proportion of the total length of the spindles counted to the weight of the muscle, and so on.

If it were to be established that a close and predictable correlation exists between the number of spindles in a given muscle and its partner, it would clearly be a considerable asset in histological and physiological investigations of this proprioceptor. It was obvious that if a result of significance was to emerge it would have to be based upon many paired counts, and from the outset we attached little importance to Morpurgo's observation or to Tower's conclusion that there was no correlation since each of these was based on only one such count.

A natural extension to the work of counting the number of spindles in a muscle is simultaneously to record their distribution within it. The large number of counts made in the limb muscles used afforded an opportunity for obtaining information about their spindle distribution to an extent which has so far only been achieved for the extraocular muscles (see Cooper & Daniel, 1949; Merrillees, Sunderland & Hayhow, 1950; Cooper, Daniel & Whitteridge, 1955).

MATERIALS AND METHODS

The muscles investigated were the rectus femoris, tibialis anticus, and Vth interosseus (forelimb) of the cat. Twenty paired counts were made of each muscle, the sex of the animals used being kept in equal proportions and litter-mates being avoided. In order to reduce the quantity of sections, the muscles were usually obtained from 0 to 21-day-old kittens, though two pairs of rectus femoris and seven pairs of Vth interosseus were obtained from adults. The muscles were fixed in a stretched condition, the entire limb being placed in Bouin's fluid, and were subsequently dissected out in 70 % alcohol. The counts were made from serial horizontal longitudinal paraffin sections cut at 10μ and stained with haemotoxylin and eosin. Some muscles were stained using the Holmes silver technique in order to study the nerve-entry and distribution of intramuscular nerve trunks.

In making the spindle counts, each section of the series was examined and the position of each spindle was noted in terms of the micrometer scale readings of the microscope's mechanical stage, the nuclear bag region being taken as the measuring point. All the counts were checked by re-counting a second time, and in some cases a third time: one third of the counts were further checked by our colleague Mr M. C. Ip.

The first muscle studied was rectus femoris. This is a pinnate muscle with a centrally placed tendon running in a sagittal plane through most of its length. The position of each spindle counted in this muscle was originally recorded in terms of the distance of its nuclear bag region from the central tendon and from the proximal and distal ends of the muscle, a method later discarded on the acquirement of a Shadowgraph contour projector. Using this apparatus a tracing was made of the projected image of every fifth section of a series, the position of the spindles being marked in directly on each tracing. This method rendered it possible to represent the distribution of spindles within any given muscle as follows: (*a*) by preparing histograms plotting spindle number against muscle length, muscle width (rectus femoris only), and muscle depth; (*b*) by preparing a projection-plan of the muscle in which the positions of all spindles present are charted within the limits of a tracing of a single representative horizontal longitudinal section.

RESULTS

Number of muscle-spindles

The results of the twenty paired counts for each muscle are shown in Table 1 and are represented graphically in Fig. 1. They provide information about the variation of the spindle content of a given muscle, and the nature of the difference in spindle content between members of a pair.

With regard to spindle content, the lowest count in rectus femoris was 77 and the highest 132, giving a range of 56 for the 40 muscles counted and a mean spindle content of 104·15 ± 13·55 (S.D.). In tibialis anticus the lowest count was 52 and the highest 89, a range of 38, and the mean content was 70·65 ± 8·76 (S.D.). In Vth interosseus (forelimb) the lowest count was 21 and the highest 31, a range of 11, and mean content of 25·1 ± 2·36 (S.D.). Though Tower (1932) also found a range of 11 for this muscle, the 10 counts made fell between 14 and 24, with a mean content of 19. We are inclined to attribute the discrepancy between these results and our own to the possibility that Tower failed to count the complete spindle content in her Bielschowsky preparations. Our own results are based on the thoroughly checked counts of 40 Vth interossei and were made with the experience of having previously counted and re-counted the spindle contents of 20 pairs of rectus femoris and tibialis anticus muscles.

The limits of difference in spindle content between members of pairs were from 1 to 9 in rectus femoris, with a mean difference of 4·4; from 1 to 8 in tibialis anticus, with a mean of 3·8; and from 0 to 4 in Vth interosseus with a mean of 2·2 (see Fig 2). We would discount Tower's difference of 8 in her one paired count of Vth interosseus (14 as to 22) for the reasons already given. The correlation coefficient (r) of the paired counts of rectus femoris is 0·94, of tibialis anticus 0·87, and Vth interosseus 0·41. The comparatively low value of r for Vth interosseus is due to the fact that although the limit of difference between each member of a pair is four spindles, and thus lower than that of the other muscles, it is by contrast a significant fraction of the spindle content. Thus while a close equivalence in spindle content between pairs of muscles containing few spindles is to be expected on functional grounds, the level of significance in terms of r is inevitably poor. An extreme case is provided by the frog toe muscle containing only one spindle from which Matthews (1931) recorded in his first single-fibre experiments. Paired counts of this muscle might well reveal the occasional presence of two or three spindles, and while this would be functionally acceptable, the value for r would be virtually zero.

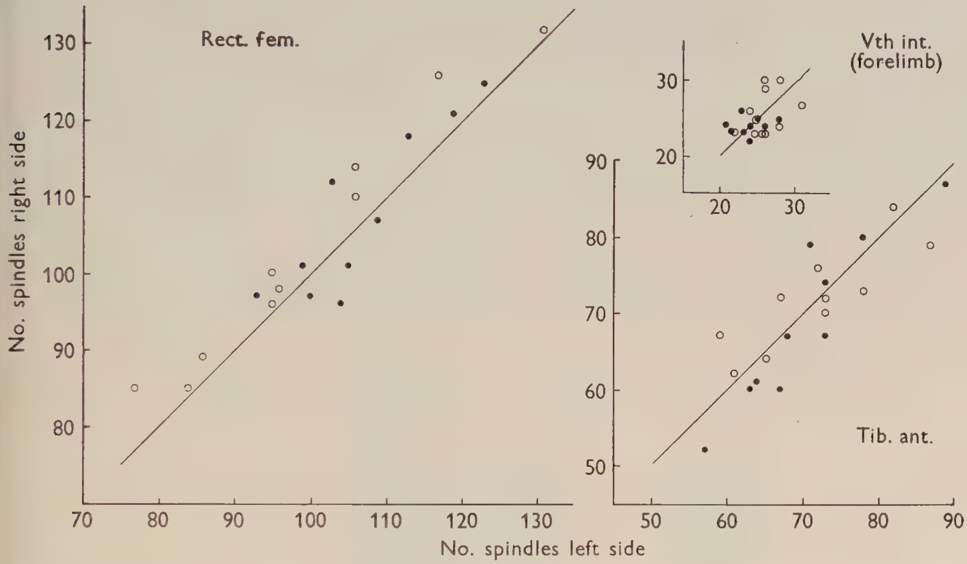

Fig. 1. Graphic representation of the spindle contents of rectus femoris, tibialis anticus, and Vth interosseus (forelimb) of cat as determined by 20 paired counts of each muscle. Each circle represents one paired count; hollow circles, females; black circles, males. Line between points shows where points would lie representing members of a pair of muscles with identical spindle content.

Bearing in mind that the main objective of these counts was to ascertain whether the spindle content of one member of a pair of identical muscles could be predicted if the content of the other member was known, the results as they stand reveal no more than the range of difference between pairs as established by 20 paired

Table 1. *Paired counts of the number of muscle-spindles in three limb muscles of the cat*

C = adult cat; K = 0 to 21-day-old kitten; R.D. = range of difference.

	Rectus femoris				Tibialis anticus				Vth Interosseus (forelimb)			
Sex	Ref. no.	Left	Right	Difference, right as to left	Ref. no.	Left	Right	Difference, right as to left	Ref. no.	Left	Right	Difference, right as to left
Female	C4	95	96	+1	K17	61	62	+1	C23	22	23	+1
	C9	77	85	+8	K24	82	84	+2	C22	26	30	+4
	K11	117	126	+9	K28	67	72	+5	C26	26	23	−3
	K19	106	114	+8	K62	73	70	−3	C28	28	24	−4
	K30	106	110	+4	K69	78	73	−5	C33	26	29	+3
	K38	86	89	+3	K71	87	79	−8	C35	28	30	+2
	K45	95	100	+5	K73	73	72	−1	K51	31	27	−4
	K49	84	85	+1	K76	59	67	+8	K54	24	26	+2
	K51	131	132	+1	K79	72	76	+4	K62	25	23	−2
	K54	96	98	+2	K82	65	64	−1	K69	26	23	−3
Totals		993	1035	+9 to +1		717	719	+8 to −8		262	258	+4 to −4
Male	K6	123	125	+2	K14	73	67	−6	C25	28	25	−3
	K9	100	97	−3	K25	57	52	−5	K52	25	25	0
	K10	119	121	+2	K43	67	60	−7	K60	22	23	+1
	K12	113	118	+5	K44	64	61	−3	K63	21	24	+3
	K25	105	101	−4	K56	68	67	−1	K73	23	26	+3
	K34	94	101	+7	K58	89	87	−2	K74	24	24	0
	K35	93	97	+4	K60	63	60	−3	K75	23	25	+2
	K41	104	96	−8	K65	73	74	+1	K77	25	25	0
	K43	109	107	−2	K67	78	80	+2	K81	24	22	−2
	K57	103	112	+9	K77	71	79	+8	K83	26	24	−2
Totals		1063	1075	+9 to −8		703	687	+8 to −7		241	243	+3 to −3
		2056	2110	R.D. = ±9		1420	1406	R.D. = ±8		503	501	R.D. = ±4
Mean spindle content and S.D.		104·15 ± 13·55				70·65 ± 8·76				25·1 ± 2·36		
Spindle content range		77 ↔ 132 = 56				52 ↔ 89 = 38				21 ↔ 31 = 11		
Mean difference between pairs		4·4				3·8				2·2		

counts. Thus if the number of spindles in the tibialis anticus muscle on one side of a cat is counted we may presume that the muscle on the other side will at the most have either eight more or eight less spindles than its partner. But the difference might well be much less, for the mean difference is 3·8 and 55 % of the pairs differed by 3 or less. We have therefore used the data obtained in deriving r and applied the regression line equation in order to obtain the best estimate of the spindle content of the left-hand muscle (y) when the spindle content of the right-hand muscle (x) is known, and vice versa. Thus the best estimate of y from a known value of x for rectus femoris is $4{\cdot}7+0{\cdot}93x$; for tibialis anticus, $12{\cdot}0+0{\cdot}84x$; and for Vth interosseus (forelimb), $14{\cdot}4+0{\cdot}43x$. Similarly, the best estimate of x for a known value of y for rectus femoris is $6{\cdot}8+0{\cdot}96y$; for tibialis anticus, $7{\cdot}1+0{\cdot}89y$; and for Vth interosseus, $14{\cdot}0+0{\cdot}4y$.

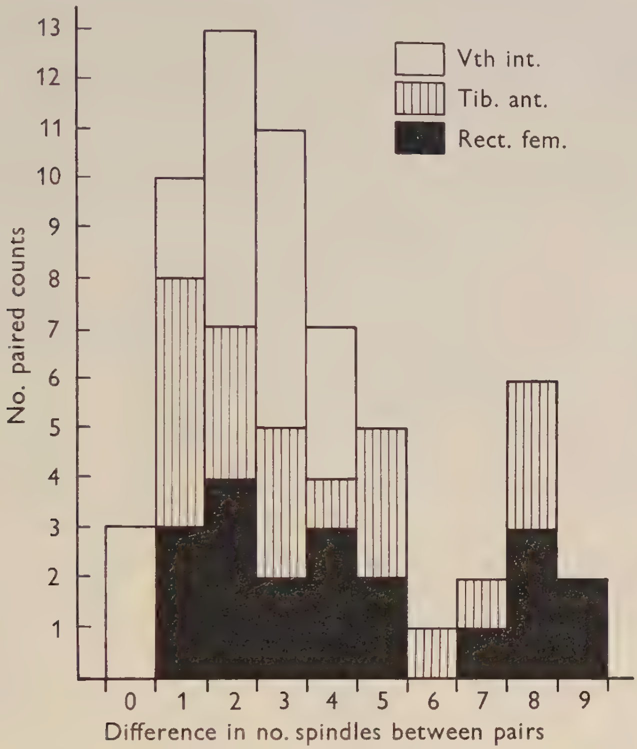

Fig. 2. Distribution of the differences in spindle content between members of pairs of the three cat muscles studied.

Within the limits of the study made, these results show that there is a close correlation between the spindle content of a pair of identical muscles from one individual. Of the total of 60 paired counts made of the three muscles studied, the difference in the spindle content of a pair ranged from 0 to 9 (Fig. 2). In three pairs of muscles (all Vth interosseus) the spindle content of each side was identical; in 24 pairs the left muscle had a greater spindle content than the right, as against 33 pairs in which the right had a greater spindle content than the left. There is thus a tendency towards asymmetry, but it is doubtful whether our relatively small sample can be regarded as a significant anatomical expression of the frequency of use of right and left limbs. We are on firmer ground in concluding that in muscles such as those we have studied, the lower the spindle content (*a*) the narrower is the range of spindle content (56, 38, and 11 in this series for muscles of mean spindle content of $104{\cdot}15 \pm 13{\cdot}55$, $70{\cdot}65 \pm 8{\cdot}76$, and $25{\cdot}1 \pm 2{\cdot}36$); and (*b*) the closer is the equivalence in content between members of a pair (mean differences of 4·4, 3·8, and 2·2, and ranges of difference of ±9, ±8, and ±4 in this series). Thus, for example, we would expect a muscle with a mean spindle content of 50 to vary in spindle content over a range of the order of 25, to have a mean difference of the order of 3 in spindle content between pairs, and a range of difference of the order of ±6. We can say little about muscles with a spindle content considerably greater than rectus femoris beyond suggesting that it is unlikely that range and difference increase in a linear fashion, and pointing out that human error would play an increasing part in the high counts that would have to be made.

Distribution of muscle-spindles

It has been frequently stated that the greatest number of spindles in a muscle occurs in its 'belly' or in the 'region of nerve-entry'. Gregor (1904) indicates nerve-entry points on his graphs of spindle number plotted against muscle length (human foetal material), but apart from this, and early work on whole mounts of muscle (e.g. Kölliker, 1862; Mays, 1884) and studies of extraocular muscles (e.g. Cooper & Daniel, 1949), no attempts have been made to correlate the distribution of spindles in a muscle with the entry and distribution of its nerve supply. Clearly the two distributions must coincide to a large extent for there is generally little intramuscular segregation of motor and sensory nerve fibres. However, the fact remains that portions of muscle taken from nerve-entry or belly regions often have a low spindle content indicating that the matter requires further elucidation. We have endeavoured to do this by preparing projection-plans of each of the muscles studied showing the distribution of the spindles and nerve supply and relating these to histograms of the spindle content in terms of muscle length, width (rectus femoris only), and depth.

Rectus femoris. This is the central muscle of the quadriceps group taking its origin from the anterior border of the acetabulum and ventral border of the ilium, and inserting on to the proximal border of the patella. The tendon of origin is prolonged within the body of the muscle in the sagittal plane as a central tendon, which terminates in the distal sixth of the muscle. An aponeurosis envelops the entire muscle, increasing in thickness towards the distal end particularly on the ventral and medial sides. A small slip of muscle is located on the medial side at approximately one-third of the distance from origin to insertion: it consists of a small group of muscle-fibres segregated from the rest by a short intramuscular tendon connected with the aponeurosis, and possibly represents the posterior head of the muscle as found in the rat. The belly of the muscle is oval in cross-section but the muscle flattens out dorso-ventrally towards each end. The muscle-fibres are arranged for the most part in a pinnate fashion and the bulk of them take their origin from the central tendon and insert on to the enveloping aponeurosis.

These features are shown schematically in Fig. 3A with the addition of the entry and distribution of the nerve supply and the distribution of the spindles. The muscle is innervated by a branch of the femoral nerve, which splits into two branches (*a* and *b*) prior to entering the muscle medially, ventrally, and obliquely in the proximal quarter just above the region of the slip. In adult cats branch *b* often enters more

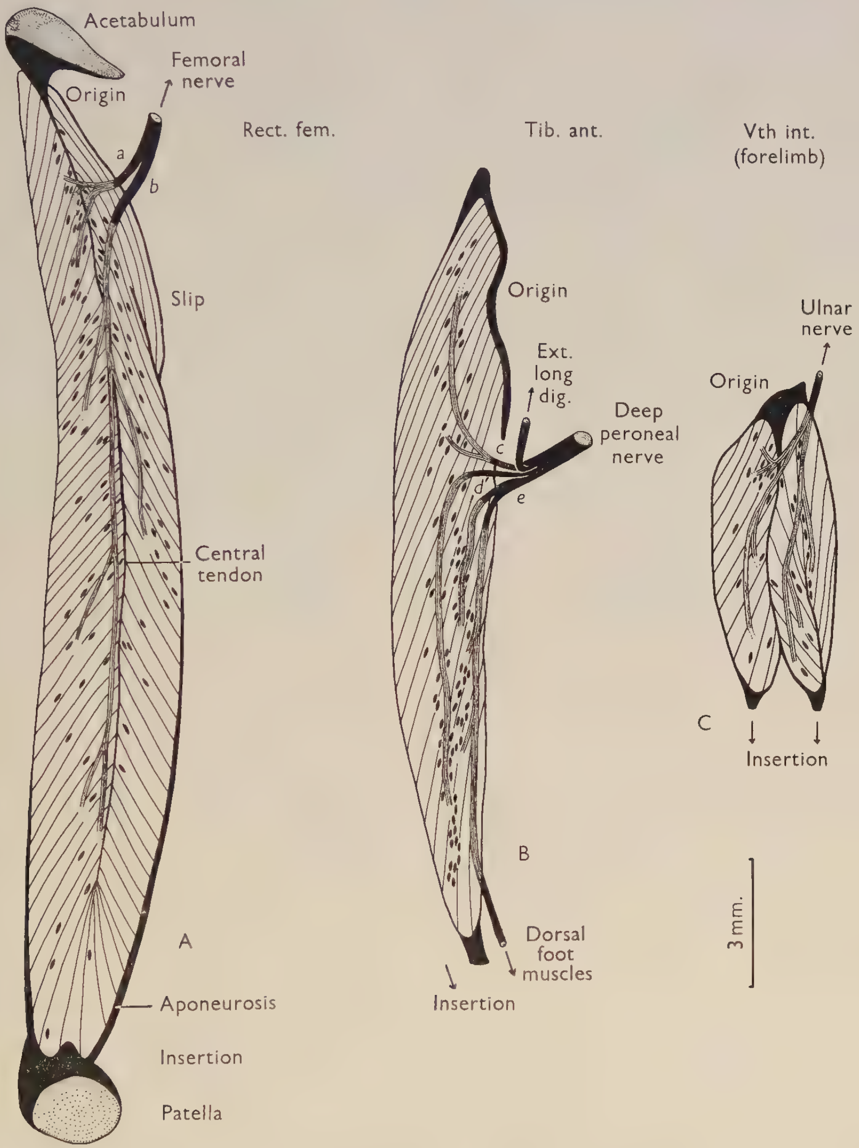

Fig. 3. Projection plans of cat rectus femoris (A), tibialis anticus (B), and Vth interosseus of forelimb (C) showing distribution of spindle and nerve supply. Spindles shown as black oval symbols; nerve branches *a*–*e* (see text) shown black up to point of entry into muscle. Based on data from right rectus femoris of kitten, K 54; right tibialis anticus of kitten K 79; and right Vth interosseus (forelimb) of young cat C 22.

distally, either in the region of the slip or just below it. The smaller and proximal branch *a* splits up into four major intramuscular trunks innervating the proximal third of the muscle: three of these trunks penetrate the central tendon to subdivide further on the lateral side. The larger branch *b* approaches the central tendon, which it penetrates after leaving one trunk on the medial side: the remaining trunks, four in number, all supply the lateral side. Branch *b* thus supplies the middle and distal thirds of the muscle. This pattern, with minor variations, held for all muscles studied. The most common variation was for the medial trunk of branch *b* to split off earlier than shown in Fig. 3A, in a few cases even prior to nerve-entry. In every distribution, however, the largest number of nerve trunks was to be found on the lateral side of the muscle.

It is clear from the distribution of the spindles as shown in Fig. 3A that the majority is located in the lateral half of the muscle. In this particular animal (K 54) there were 58 spindles in the lateral half as compared with 40 in the medial half. To a greater or lesser extent this is always the case, but more pronounced features are the concentration of spindles in the ventral half of the muscle and the preponderance of spindles in the proximal as compared with the distal half of the muscle. Histograms of the spindle distribution emphasize these features, and when the histograms for each member of a pair of recti from one individual are compared there is seen to be a close similarity in distribution on the two sides (see Fig. 4). A comparison of histograms prepared of the spindle distribution in six pairs of recti suggests that only minor variations occur between different individuals. If the muscle length histograms for rectus femoris in Fig. 4 are compared with the spindle-number muscle-length graph prepared by Gregor (1904, fig. 18) for the rectus femoris of a human foetus, it is clear that in both cat and man the majority of the spindles in this muscle lies in its proximal half. The nerve-entry points on Gregor's graph, and his text, also suggest that the nerve entry and supply of the muscle are similar. The comparison is somewhat marred by the fact that Gregor did not examine that part of the muscle corresponding to the proximal third of the femur.

We may summarize these results as follows. The nerve supply of the rectus femoris muscle of the cat enters the proximal quarter of the muscle on the ventro-medial side and is distributed intramuscularly primarily on the ventro-lateral side. The spindles are distributed in greater quantity in the ventral half of the muscle than the dorsal, more in the proximal half than the distal, and more in the lateral half than the medial. The greatest concentration of spindles occurs in the proximal ventro-lateral eighth of the muscle.

Tibialis anticus. This muscle lies superficially on the lateral side of the shank, its inner concave surface clothing the underlying extensor longus digitorum. Its origin from tibia and fibula extends for about one-third of its length on the medial side, and its insertion is by way of a long tendon, which ultimately attaches on to the outer surface of the first metatarsal. The muscle fibres run diagonally and medio-laterally as shown in Fig. 3B. The innervation is supplied by the deep peroneal nerve, which enters the muscle on the medial side in the middle third region with respect to depth, and approximately at the junction of the proximal and middle third regions with respect to length. Prior to entering the muscle, the nerve divides into four branches, three of which enter the tibialis anticus (*c*, *d*, *e*), while the fourth innervates the extensor longus digitorum (see Fig. 3B). The proximal third of the muscle is innervated by branch *c*, the rest by *d* and *e*; branch *e* splits into two intramuscular trunks, the most medial of which, after contributing to the innervation of

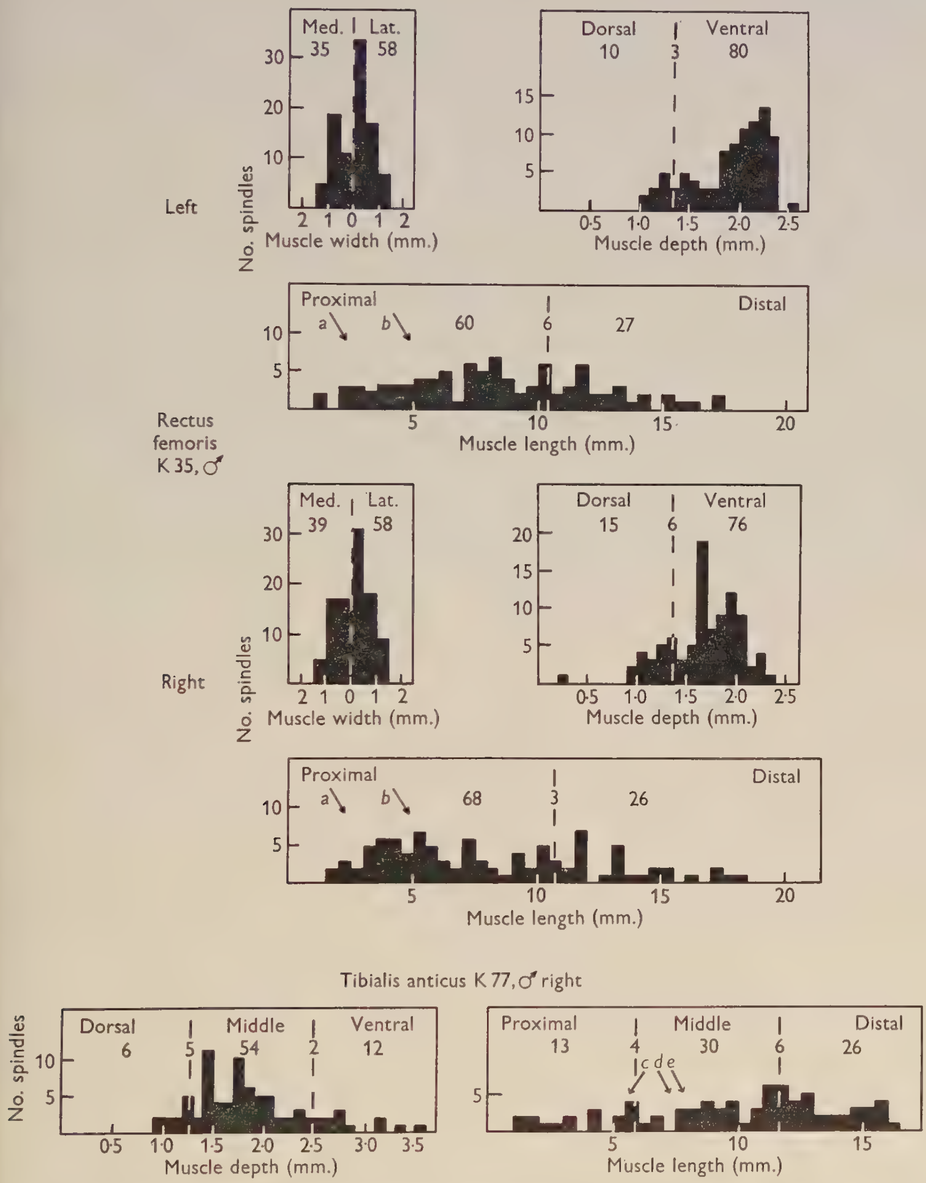

Fig. 4. Distribution of spindles in cat rectus femoris and tibialis anticus muscles as illustrated by histograms plotting spindle number against muscle width (rect. fem. only), depth and length. The distribution is shown for one pair of recti from kitten K 35, and for the right tib. ant. from kitten K 77. *a*, *b*: proximal and distal branches of nerve supplying rect. fem. (cf. fig. 3A); *c*, *d*, *e*: nerve branches supplying tib. ant. (cf. fig. 3B). Arrows indicate nerve-entry points (tips of arrows) and direction of entry (angle of arrows).

tibialis anticus, emerges at the distal end of the muscle with the tibialis artery to travel to the foot, where it innervates the dorsal muscles.

The distribution of spindles is shown in Fig. 3B, a projection-plan of the muscle from the right side of kitten K 79, and in Fig. 4, which illustrates the distribution in the right-hand muscle of kitten K 77 by means of histograms in terms of muscle depth and length. The spindles are seen to be distributed in the neighbourhood of the intramuscular nerve trunks, the greatest concentrations being in the middle third of the muscle with respect to depth (i.e. the level of nerve-entry) and in the middle and distal thirds with respect to length. The portion of the muscle containing the least number of spindles is the dorsal and proximal ninth. A comparison of our muscle-length histograms with the spindle-number/muscle-length graphs prepared by Gregor (1904, figs. 34, 35) for the human foetal tibialis anticus, suggests a similar spindle distribution in this respect in both cat and man.

Vth interosseus (*forelimb*). This is a small stout muscle lying on the palmar surface of the fifth digit of the paw. It is double-bellied and has a single origin, two tendons of insertion, and a pinnate arrangement of muscle fibres (see Fig. 3C). The innervation is supplied by the deep palmar branch of the ulnar nerve, which enters the muscle from the medial side at the proximal end at the level of the middle third region with respect to depth. After entry the nerve branches into two intramuscular trunks, which supply the medial and lateral halves of the muscle. As in the other muscles described, the spindles are distributed in the neighbourhood of the intramuscular nerve trunks. Histograms of the distribution show that most of the spindles are located in the proximal and middle thirds of the muscle with respect to length, and the middle and ventral thirds with respect to depth.

DISCUSSION

The fact that the spindle contents of a pair of identical muscles from one individual are closely equivalent is not an unexpected finding, but the demonstration in the muscles studied that this is so, together with the establishment of the limits of difference and prediction of difference formulae have useful applications, of which we are already taking advantage in other work. It may eventually be possible from the result of a single count of any given muscle, to predict its range and mean spindle content, and range and mean difference between members of pairs, but the data from a large quantity of paired counts of many muscles would be necessary before this could be realized. Hines & Tower (1928) were of the opinion that the number of spindles in a given muscle was approximately constant, but it has been clear for some time that the spindle content does in fact vary in different individuals (see, for example, the results of Voss, 1937, for human lumbricales muscles; and Merrillees *et al.* 1950, for the human superior oblique muscle). It is also evident that the spindle content of a muscle does not necessarily bear any relation to its size. That the spindle content of a muscle varies is, however, sometimes ignored. Thus, for example, Hagbarth & Wohlfart (1952) relate a spindle count of 57 for the tibialis anticus muscle of one cat with the afferent fibre diameter spectrum of another on the assumption that the spindle content of the muscle in both cats was the same. Their deductions are accepted by Granit (1955) but the basis for them is clearly invalid; as our results show, the spindle content of the muscle in the operated cat could have been anything between 52 and 89, the probability being 67·5 % that it lay between 62 and 79.

The use of kitten muscles for a large number of the counts might be objected to in view of the possibility of a post-natal increase in the number of spindles. However, studies of the development of spindles (e.g. Tello, 1917; Cuajunco, 1927) make it clear that the spindle content of a muscle is established before birth and that post-natal morphogenesis is simply concerned with further changes (increase in length, elaboration of capsule, etc.) in spindles already present (Kalugina, 1956; Zelená, 1957). Voss (1937) found no significant increase in the spindle content of the lumbricales muscles of the human hand in counts made on newly-born and adult material, and this is also our experience in counts of kitten as compared with adult muscles (rectus femoris and Vth interosseus, forelimb).

Our counts of spindles were made on the assumption that the receptors consisted of a single encapsulated region situated equatorially between two poles in accordance with the classical descriptions. However, Cooper & Daniel (1956) have described the occurrence of 'tandem' spindles in human hand and neck muscles in which several capsular regions occur successively in series, and Gray (1957) has described similar 'spindle systems' in the ext. long. dig. IV muscle of the frog. Tandem spindles with two, and occasionally three, successive capsular regions have been observed by Barker and Ip (unpublished) in the rectus femoris muscle of the cat and their results at present suggest that about 20 % of the spindles in this muscle are of this type. In longitudinal sections, as used in this study, it is impossible to decide whether or not two or three closely associated capsular regions belong to separate spindles or to one tandem spindle. In view of these observations, our counts of spindles should more accurately be regarded as counts of encapsulated sensory regions of spindles, a proportion of which are arranged in tandem.

Previous studies of spindle distribution have been made almost exclusively on muscles cut in serial transverse section. This permits variations in spindle length to be recorded and providing that due attention is paid to the location of each spindle within the muscle, projection-plans of spindle distribution can be prepared. These vary in their usefulness from the clear presentations of Merrillees *et al.* (1950) to the misleading figures of Hagbarth & Wohlfart (1952), which show the distribution of spindles not, as claimed, within the muscle containing them, but simply on the grid used by the authors to record them. While nerve-entry and distribution could be mapped from a transverse section study it would be a laborious undertaking and has not been attempted in the studies made. To this limitation of the method must be added the fact that it can only work well with muscles whose fibres are orientated parallel with the longitudinal axis. By contrast longitudinal sections allow for projection-plans to be prepared, which show muscle-fibre direction, distribution and orientation of spindles, and the entry and distribution of the nerve supply. Moreover, counting, which necessarily goes hand in hand with distribution, is more rapid with spindles cut in longitudinal than in transverse section.

Our distribution results show clearly that in the muscles studied the spindles are distributed in the neighbourhood of the major intramuscular nerve trunks. The same conclusion was drawn by Gregor (1904) in his monumental study of the number and distribution of spindles in 51 muscles of the human foetus. The pattern of the intramuscular nerve supply of a given muscle is fairly constant, minor variations occurring in different individuals but to an insignificant extent between members of a pair of identical muscles from the same individual. Spindle distribution follows accordingly, so that its pattern in each member of a pair of muscles from one individual is very similar, as Morpurgo (1897) observed in the rat's sartorius. However, since the distribution of major intramuscular nerve trunks follows a different pattern in different muscles, concentrations of spindles do not necessarily occur in 'regions of nerve-entry' or in the 'belly' of a muscle as has often been stated. 'Nerve-entry' concentrations are likely to occur only where there is a rapid subdivision of the intramuscular supply in this region, and 'belly' concentrations only where there is a supply of one or more intramuscular trunks to this region. This feature follows naturally from the manner in which spindles are formed in embryo muscle. It would appear from Tello (1917) that the pattern of the intramuscular nerve supply is established mainly by the extrafusal motor fibres. Spindle formation is initiated when a primary nerve fibre leaves an intramuscular nerve trunk and comes into association with a bundle of myotubes nearby. The characteristic spindle nerve trunks are formed when other nerve fibres destined for the spindle (intrafusal motor fibres, 'accessory' fibres, and in some cases secondary fibres) arrive later and follow the pathway laid down by the primary fibre. Thus providing the primary fibres are distributed generally throughout the intramuscular nerve supply, the distribution of spindles in the neighbourhood of the major intramuscular nerve trunks is bound to follow. With most muscles this would appear to be so but the extraocular muscles are exceptional in that there is a high degree of segregation of the extrafusal motor and spindle supply in the intramuscular trunks, the former being restricted to approximately the middle third of the muscles and the latter either mainly to the proximal third (Cooper & Daniel, 1949, human inferior rectus) or to both proximal and distal thirds of the muscles (Merrillees, *et al.* 1950, human medial and superior recti, and superior oblique).

SUMMARY

A study of the number and distribution of muscle-spindles in twenty pairs each of mm. rectus femoris, tibialis anticus, and Vth interosseus (forelimb) of the cat has shown that:

1. The spindle content of a muscle varies in different individuals, as has been established by other workers. In the 40 counts made of each muscle the spindle content fell between 77 and 132 in rectus femoris (range 56: mean 104·15 ± 13·55 S.D.); 52 and 89 in tibialis anticus (range 38: mean 70·65 ± 8·76 S.D.); and 21 and 31 in Vth interosseus (range 11: mean 25·1 ± 2·36 S.D.).

2. There is a close correlation between the number of spindles in a given muscle and its partner on the opposite side of the animal. In this series the difference in spindle content of a pair ranged from nil to 9. From a statistical treatment of the results, formulae are derived for obtaining the best estimate of the spindle content of the left member of a pair when the content of the right member is known, and vice versa.

3. In these muscles the lower the spindle content, the closer was the equivalence in content between members of a pair (mean differences of 4·4, 3·8, and 2·2, and ranges of differences of ±9, ±8, and ±4 for rect. fem., tib. ant., and Vth int. respectively).

4. Of the 60 paired counts made, the spindle contents of three pairs (all Vth interosseus) were identical, and the right muscle had a higher spindle content than the left in nine more pairs than vice versa.

5. Since it is probable that some of the spindles counted were of the tandem type, our counts should be regarded as counts of encapsulated sensory regions of spindles, a proportion of which are arranged in tandem.

6. Projection-plans of each muscle studied have been prepared showing muscle-fibre direction, distribution and orientation of spindles, and the entry and distribution of the nerve supply. The spindle distribution is also illustrated by histograms of the spindle content in terms of muscle length, width (rect. fem. only), and depth. It is demonstrated that the spindles in a muscle are distributed in the neighbourhood of the major intramuscular nerve trunks. Since the distribution of these trunks follows a different pattern in different muscles, concentrations of spindles do not necessarily occur in 'regions of nerve-entry' or in the 'belly' of a muscle as has often been stated.

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THE COMPARATIVE ANATOMY OF THE RESPIRATORY PATHWAYS IN THE DOMESTIC FOWL (*GALLUS DOMESTICUS*), PIGEON (*COLUMBA LIVIA*) AND DOMESTIC DUCK (*ANAS PLATYRHYNCHA*)

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INTRODUCTION

This investigation was carried out to provide a comparative anatomical picture of the respiratory system in the domestic fowl, pigeon and duck. It will be used as the basis of a functional study of avian respiration, in which cineradiography will be the principal technique.

MATERIALS AND METHODS

Rubber latex casts of the respiratory system of twenty fowl, twelve pigeons and six duck were used in the present study. The birds were killed by a rapid intravenous injection of Nembutal, and latex was injected into the system, via the trachea, at about 10 lb/sq. in.

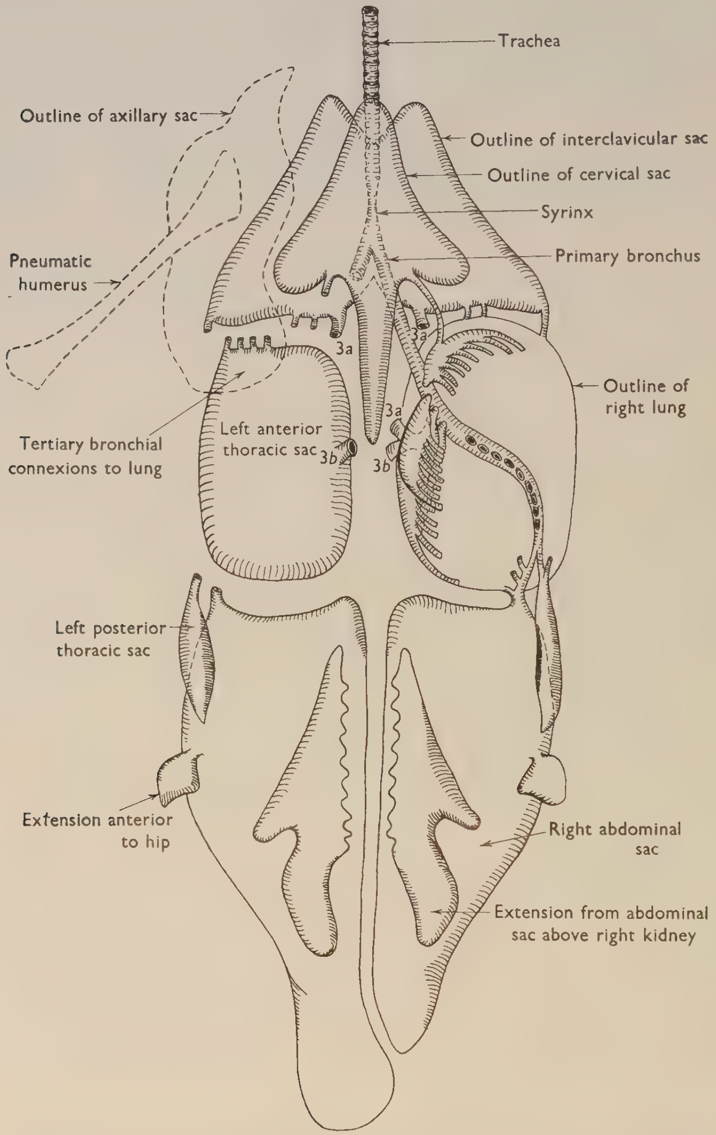
The weakest points in the system were the two large abdominal air sacs which might rupture before the most remote extensions of some of the other sacs had been filled. To reduce this risk the body of the bird was laterally compressed before injection was started, and during the whole of the injection period this manual pressure was maintained until it was clear that the abdominal sacs were about to burst. At this point injection was stopped, the trachea ligated and the bird placed in a bath of concentrated hydrochloric acid. After 2 or 3 days the cast was transferred to a bath of warm soapy water and sprayed with water to remove the adherent debris.

Rubber latex gives an extremely accurate cast of the system, provided that an adequate injection pressure is used. It stands a considerable amount of movement and bending, so that the complicated network of interconnected tubes in the lung and their delicate connexions to the air sacs can be studied without fear of breaking them. The cast can be cut easily with scissors to expose tertiary-bronchial relations with secondary bronchi deep inside the lung, or parts can be removed to give a clear picture of what remains. The latex can be made any colour by adding liquid dyes. For photographic purposes a light colour is best, and yellow was used for all the casts in this study.

THE STRUCTURE OF THE RESPIRATORY SYSTEM IN THE DOMESTIC FOWL (*GALLUS DOMESTICUS*)

Throughout this description, for the most part, the term 'connected to' has been used instead of such terms as 'gives rise to' or 'receives' because it is felt that no functional implication as to the direction of air flow can be read into the former, whereas it could be in the latter.

The respiratory pathways in a bird consist of—trachea, syrinx, primary bronchi (mesobronchi), secondary bronchi, tertiary bronchi (parabronchi), air capillaries, air sacs and pneumatic bones. *The lungs* occupy the cranio-dorsal part of the body


Trachea
Outline of axillary sac
Outline of interclavicular sac
Outline of cervical sac
Syrinx
Pneumatic humerus
Primary bronchus
3a
3a
Outline of right lung
3a
Tertiary bronchial connexions to lung
Left anterior thoracic sac
3b
3b
Left posterior thoracic sac
Extension anterior to hip
Right abdominal sac
Extension from abdominal sac above right kidney

Text-fig. 1. Connexions between lungs and air sacs in the fowl (dorsal view). The left lung has been removed to show its connexions to the left anterior thoracic sac. The left axillary sac and its extension into the humerus are shown as a dotted outline superimposed on top of the left side of the interclavicular sac. In the right lung the secondary bronchi are shown in their correct orientation so that the posterior group is at right angles to the plane of the paper. The two large ducts (marked 3*a* and 3*b*) with a common origin from no. 3 anterior dorsal secondary bronchus connect directly with the interclavicular and anterior thoracic air sacs on their own side.

cavity, and lie immediately above the heart. The dorsal surface of each is deeply grooved by ribs 4, 5, 6 and 7. Each lung contains one primary bronchus, two groups of secondary bronchi, a great many tertiary bronchi and innumerable air capillaries, together with a pulmonary and bronchial blood supply. *The trachea* is supported throughout its length by complete cartilaginous rings, which become bony with age. It enters the body cavity to the left of the crop and bifurcates at the syrinx into two primary bronchi. *The syrinx* is situated at the base of the trachea where it is surrounded by the interclavicular sac. It contains the vocal cords inside and has several associated muscles outside.

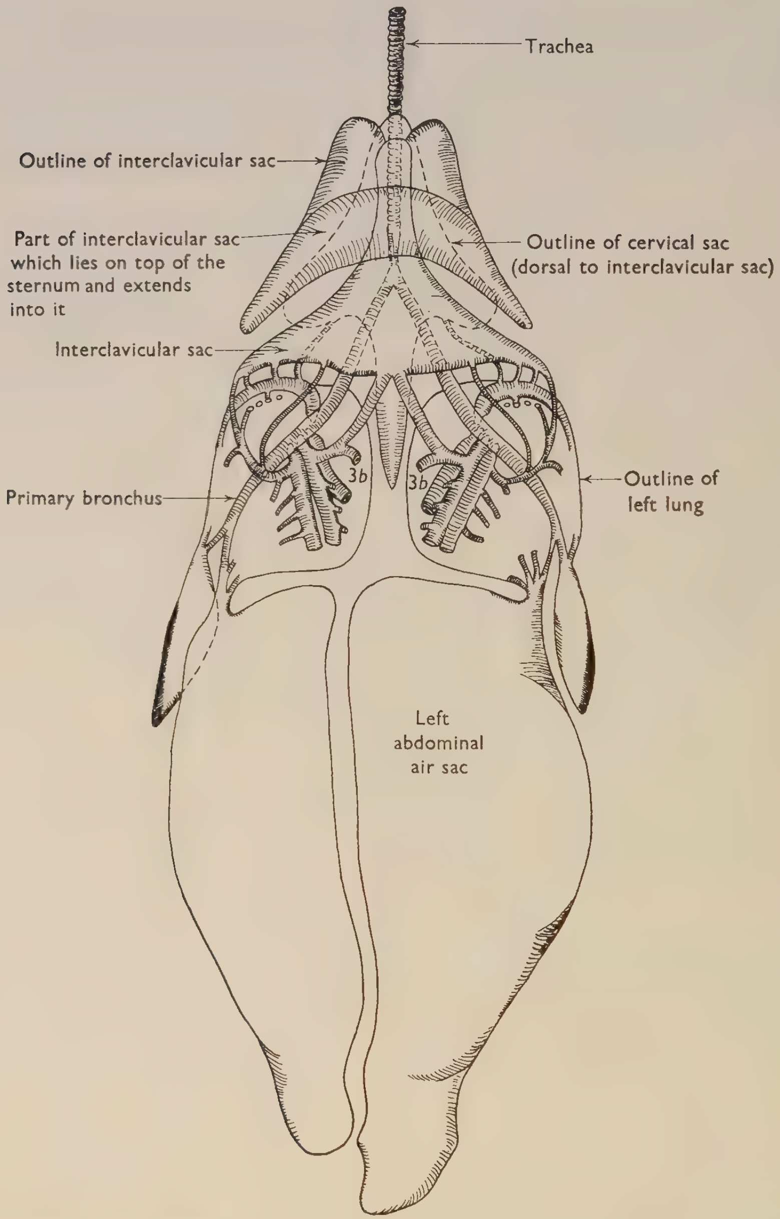
Primary bronchi (mesobronchi)

These originate in the syrinx and one runs through each lung (Text-figs. 1–5). Each gives rise to two groups of secondary bronchi, as well as to many tertiary bronchi directly. A primary bronchus enters each lung on its medial side at the junction of its anterior and middle thirds, more or less at right angles to the lung. Immediately after its point of entry it gives rise to four large dorsal secondary bronchi (Text-figs. 3–5; Pl. 3). It then describes a rather flattened horizontal 'S' bend in its passage through the lung. The anterior part thus runs from the medial towards the lateral side, whilst the posterior part runs parallel to the lateral wall of the lung and is separated from it by a thin layer of tertiary bronchi. As well as being in the shape of a flattened 'S', the primary bronchus has a slightly greater diameter over its central portion (diameter $4\frac{1}{2}$ mm.) than it has at either end. (Anterior end diameter 4 mm., posterior end diameter 1 mm., see also Table 2.) This very slight increase in diameter of the primary bronchus in no way constitutes the 'vestibule' which played such an important part in the descriptions of Huxley (1882), Fischer (1905), Brandes (1924), Vos (1934) and Hazelhoff (1951). In fact the casts used in this study confirm a recent observation by Payne & King (1959) that probably there is no vestibule at all in the lungs of *Gallus domesticus*.

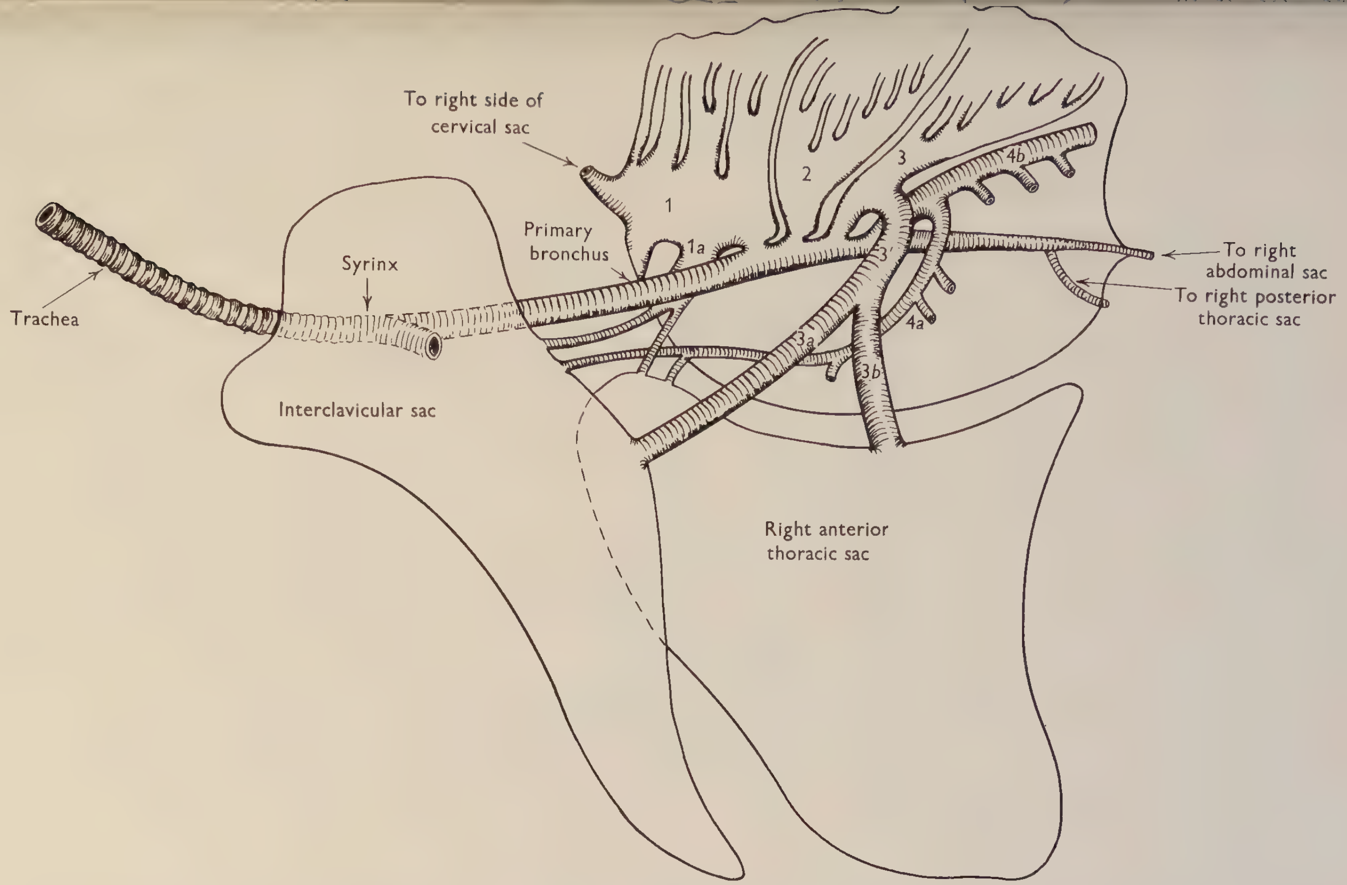
Table 1. *To show how subsidiary bronchi are connected to the various surfaces of the primary bronchus in the fowl*

Surface of primary bronchus	Anterior or posterior part of primary bronchus	Direct connexions from primary bronchus to:	
		Secondary bronchi	Tertiary bronchi
Dorsal	Anterior	4 large anterior dorsal secondary bronchi	None
	Posterior	8–9 smaller posterior dorsal secondary bronchi	None
Ventral	Anterior	None	None
	Posterior	7–8 posterior ventral secondary bronchi	None
Lateral	Anterior	None	None
	Posterior	None	Several (about 20)
Medial	Anterior	None	None
	Posterior	None	None

Throughout the entire length of the primary bronchus no ducts of any sort are connected to its medial surface. Between the last secondary bronchus of the anterior group and the first of the posterior group, the primary bronchus is free, on all sides, from any connexions to subsidiary ducts over a distance of about 4 mm. (Text-figs. 4, 5 and Table 2).


Trachea
Outline of interclavicular sac
Part of interclavicular sac which lies on top of the sternum and extends into it
Outline of cervical sac (dorsal to interclavicular sac)
Interclavicular sac
3b
3b
Primary bronchus
Outline of left lung
Left abdominal air sac

Text-fig. 2. Connexions between lungs and air sacs in the fowl (ventral view). Both anterior thoracic sacs have been removed. The row of four small holes in the antero-lateral corner of each lung represent tertiary-bronchial connexions with the anterior thoracic air sac. The large duct (3*b*) from no. 3 anterior dorsal secondary bronchus also connects with the anterior thoracic air sac (cf. Text-fig. 3).


To right side of cervical sac
Trachea
Syrinx
Primary bronchus
1
1a
2
3
3'
3a
3b
4a
4b
To right abdominal sac
To right posterior thoracic sac
Interclavicular sac
Right anterior thoracic sac

Text-fig. 3. Connexions between right lung and air sacs in the fowl (medial view). The cervical sac has been removed. Only two tertiary bronchi are shown connecting the lung to the interclavicular and anterior thoracic sacs. In fact there are about six or eight (Table 4). The large duct which connects no. 3 anterior dorsal secondary bronchus to these same two sacs has been greatly elongated for the sake of clarity. In a fully grown cockerel this duct (3′) bifurcates almost immediately after its origin from the parent duct. Each branch (3*a*, 3*b*) then runs for only 7 or 10 mm. before connecting with the appropriate sac.

Secondary bronchi

There is an anterior and a posterior group of secondary bronchi which are joined together by a great many tertiary-bronchial arcades.

The *anterior group* consists of four ducts, each being connected to the dorsal surface of the primary bronchus immediately after it enters the lung. Their points of connexion lie directly one behind the other, so that they may be numbered 1, 2, 3 and 4 (Text-figs. 3–5; Pl. 3). Each one is connected to several tertiary bronchi (Table 3) which occupy a definite region of the lung, although there are many interconnexions between these tertiary bronchi. Secondary bronchi of this group are connected to tertiary bronchi only on their lateral surfaces (i.e. on the surfaces facing into the centre of the lung). The medial surfaces of these ducts constitute the medial surface of the lung itself. Some of these secondary bronchi are connected directly to air sacs (Text-figs. 3, 4).

Table 2. *Dimensions of the various ducts which make up the avian lung. (The diameters are approximations and refer to a full-grown fowl)*

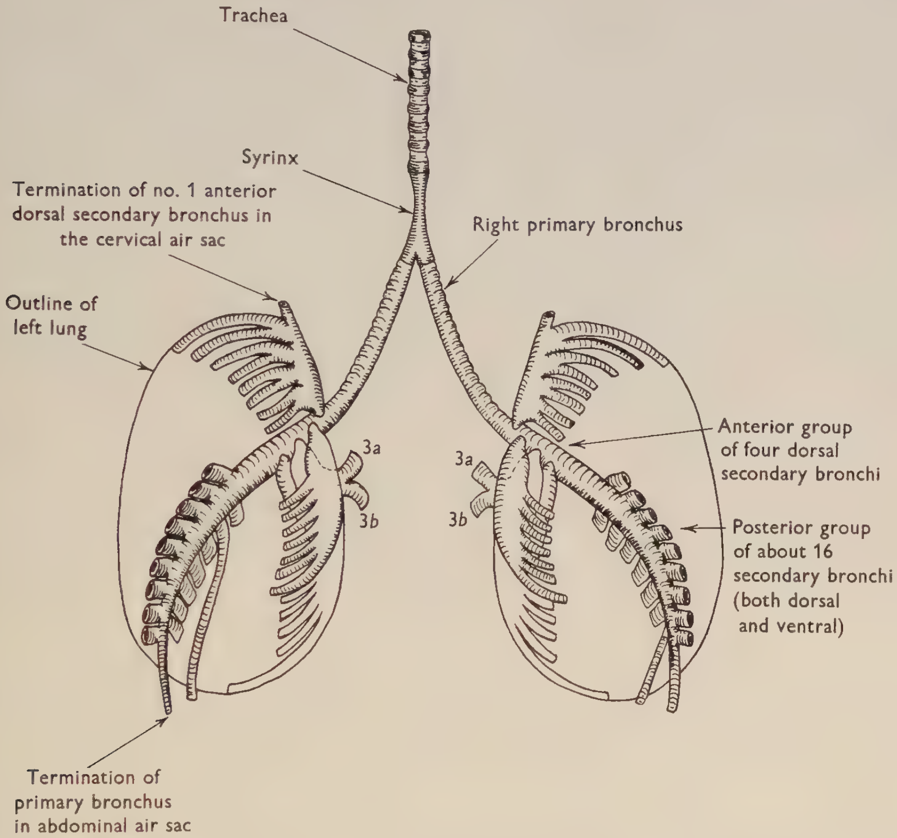
Duct	Maximum diameter in mm. (except for capillaries)
Primary bronchus	
Anterior end	4
Middle	4½
Posterior end	1
Anterior dorsal secondary bronchi	
At their points of origin from the primary bronchus, nos. 1–4	2
At their points of maximum diameter	
No. 1	8
No. 2	5
No. 3	5
No. 4	3
Posterior secondary bronchi	3
Tertiary bronchi	1
Air capillaries	10μ

Table 3. *To show connexions between anterior dorsal secondary bronchi, tertiary bronchi and air sacs in the fowl*

Anterior dorsal secondary bronchus	Approx. no. of tertiary bronchi that are connected with its lateral surface	Region of lung occupied by these tertiary bronchi	Direct connexions with air sacs
No. 1	60	Anterior 1/3 of lung	Terminates in cervical sac
No. 2	30	Central 1/3 of lung	None
No. 3	25	Posterior 1/3 of lung	A large duct leaves this secondary bronchus and bifurcates. One branch connects with the interclavicular sac, whilst the other branch connects with the anterior thoracic sac
No. 4	25	Entire ventral surface of lung	None

Secondary bronchi of this anterior dorsal group are the most obvious in the lung, and presumably represent the 'ventral secondary bronchi' of Fischer (1905), Schulze (1911), Brandes (1924) and Hazelhoff (1951), and the 'ventro-medial' group of King (1956). However, as they connect to the dorsal surface of the primary bronchus and then, for the most part, run dorsally to give rise to dorsally running tertiary bronchi, it is difficult to see how they ever came to be called 'ventral' (Pl. 3).

At their points of connexion to the primary bronchus, the anterior group of secondary bronchi have a diameter of about 2 mm. After this narrow neck they


Trachea
Syrinx
Termination of no. 1 anterior dorsal secondary bronchus in the cervical air sac
Right primary bronchus
Outline of left lung
3a
3a
3b
3b
Anterior group of four dorsal secondary bronchi
Posterior group of about 16 secondary bronchi (both dorsal and ventral)
Termination of primary bronchus in abdominal air sac

Text-fig. 4. To show the distribution of primary and secondary bronchi within the lungs of the fowl (dorsal view). In this diagram, whilst the anterior group of dorsal secondary bronchi is shown correctly from the dorsal aspect, the posterior group is shown dorsomedially in order to illustrate the ventral secondary bronchi of this group. From the dorsal aspect the four anterior dorsal secondary bronchi are superimposed on one another, although their points of connexion to the primary bronchus lie one behind the other (cf. Text-figs. 3, 5). Note the space between the two groups of secondary bronchi in which no ducts are connected to the primary bronchus. The left lung shows a direct connexion between no. 2 posterior ventral secondary bronchus and the posterior thoracic air sac (cf. Pl. 4). This was the arrangement in all casts used in the present study. The right lung shows an alternative arrangement, in which the primary bronchus bifurcates and is connected directly to both the posterior thoracic and abdominal sacs. This is frequently said to occur (Fischer, 1905; Juillet, 1912; Brandes, 1924), but would appear not to be the case.

dilate to become the largest ducts in the entire lung (Text-fig. 3). No. 1 anterior dorsal secondary bronchus has a maximum diameter of about 8 mm. and is the largest duct anywhere in the entire respiratory system (Table 2).

The *posterior group* consists of approximately sixteen ducts, eight or nine of which are connected to the dorsal surface of the primary bronchus, and seven or eight are connected to its ventral surface. They meet the primary bronchus at an angle of about 60° (Text-figs. 4, 5; Pl. 4). These secondary bronchi are much less obvious than those of the anterior group, and are connected to tertiary bronchi on both their medial and lateral sides. Each has a very slight constriction at the point where it connects to the primary bronchus, but for the most part they maintain a uniform diameter of about 3 mm. The second posterior ventral secondary bronchus terminates in the posterior thoracic sac, and is the only secondary bronchus of the posterior group in direct connexion with an air sac. Adjacent posterior secondary bronchi are connected together by very short tertiary bronchi, they are connected to the anterior group of secondary bronchi by the tertiary-bronchial arcades, and they also give rise directly to air capillaries.

Tertiary bronchi

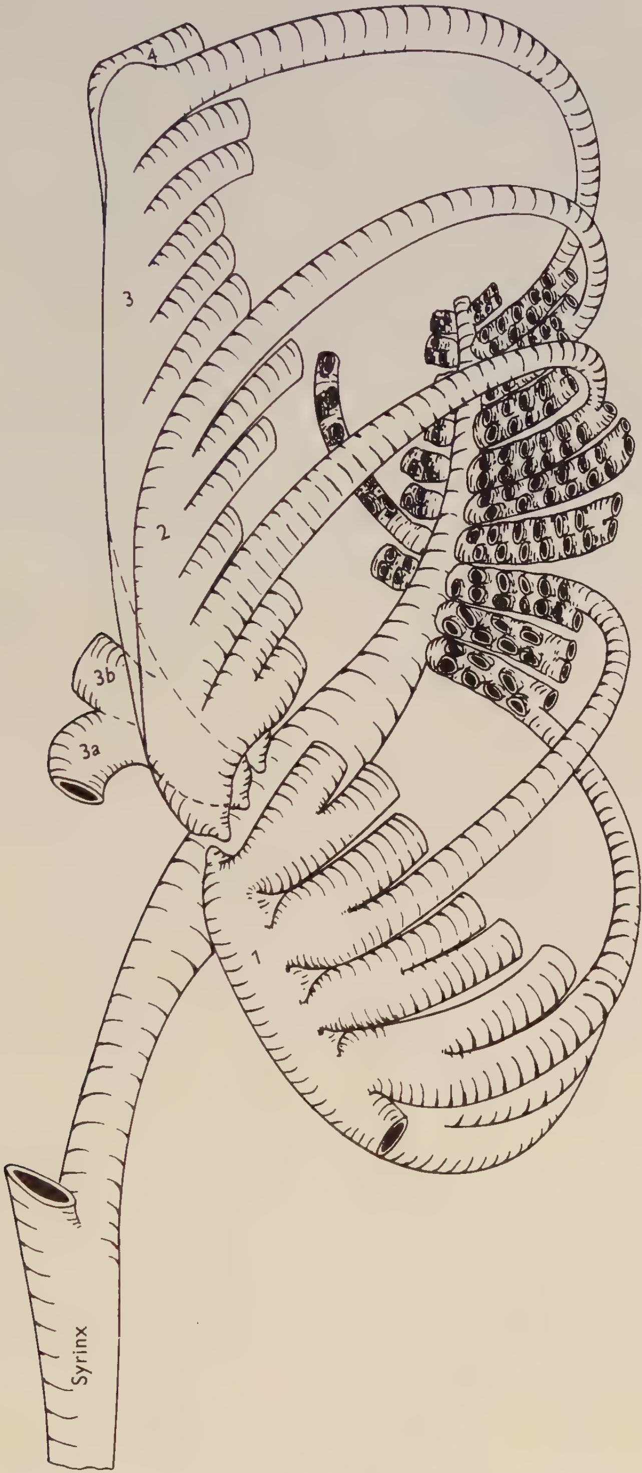
These are ducts of about 1 mm. in diameter which make up the bulk of each lung (Text-fig. 5; Pl. 2) and which, for the most part, form many arcades linking together the two groups of secondary bronchi. They are distributed throughout the entire depth of the lung, and between them there are many interconnexions. In addition to the arcades, several tertiary bronchi on the lateral side of the lung connect the posterior lateral wall of the primary bronchus to the ventral branches of no. 1 anterior dorsal secondary bronchus, and also to the posterior thoracic and abdominal air sacs. These are the twenty tertiary bronchi referred to in Table 1.

Tertiary bronchi give rise to the air capillaries (diameter about 10μ), which radiate out from small recesses in their walls and become intimately associated with blood capillaries.

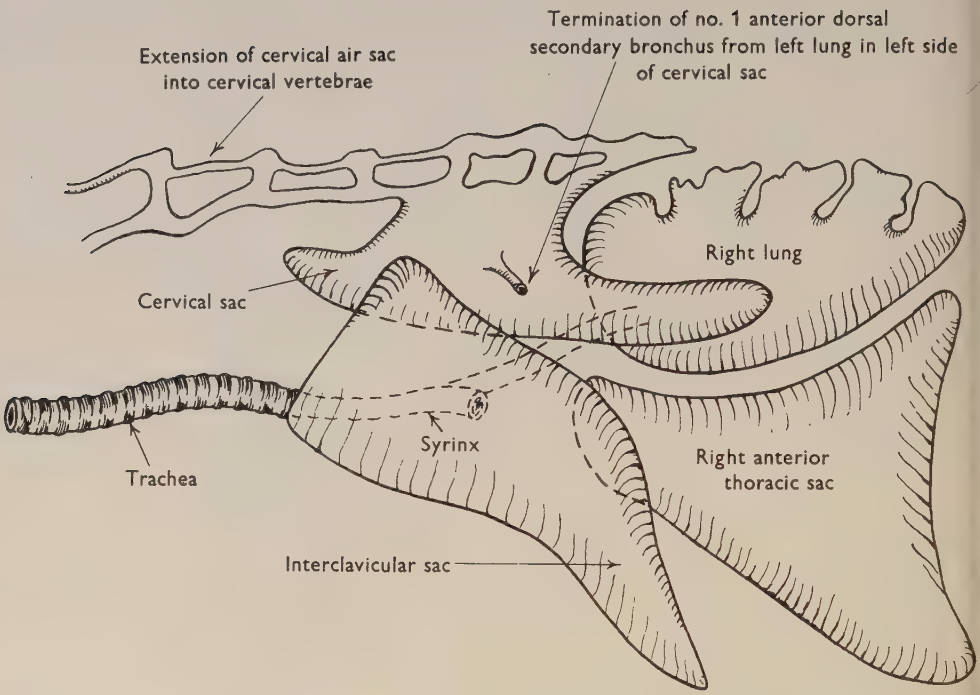
Air sacs

There are eight separate and distinct air sacs. Some are paired whilst others are single midline structures, although these developed in the embryo as paired sacs. Some have extensions into pneumatic bones and others have extensions in the form of subsidiary sacs. The air sacs are as follows: cervical (single), interclavicular (single), anterior thoracic (paired), posterior thoracic (paired) and abdominal (paired). The axillary sacs, which give origin to the extensions into each humerus, are themselves extensions of the interclavicular sac (Text-fig. 1; Pl. 1).

Air sacs are very thin-walled structures, with a poor blood supply, all of which are connected to tertiary bronchi in the lung (i.e. to tubes of about 1 mm. in diameter). Some sacs (interclavicular and anterior thoracic) are connected to the lung by a larger duct as well as by tertiary bronchi. In this case a branch from no. 3 anterior dorsal secondary bronchus bifurcates and enters these two sacs directly (Text-fig. 3). No other sac is connected to the lung by a large duct. When the primary bronchus enters the abdominal sac it is about the same size as a tertiary bronchus. There are no connexions directly between air sacs, all of which, to varying degrees, are in contact with organs of the body cavity (Table 4; Text-fig. 6).



Text-fig. 5. To show tertiary-bronchial arcades connecting together the anterior and posterior groups of secondary bronchi in the lung of the fowl (anterior dorsomedial view).


Extension of cervical air sac into cervical vertebrae
Termination of no. 1 anterior dorsal secondary bronchus from left lung in left side of cervical sac
Cervical sac
Right lung
Trachea
Syrinx
Right anterior thoracic sac
Interclavicular sac

Text-fig. 6. Relationship between right lung and cervical interclavicular and anterior thoracic air sacs in the fowl (medial view).

Pneumatic bones of the fowl

The following bones have been found to receive extensions from air sacs:

Bone	Sac
Sternum (mostly around the rostrum, not in the keel)	Interclavicular
Humerus	Axillary
Coracoid	Interclavicular
Cervical and thoracic vertebrae	Cervical
Lumbar vertebrae	Abdominal

THE STRUCTURE OF THE RESPIRATORY SYSTEM IN THE PIGEON (*COLUMBA LIVIA*)

The arrangement of the lungs and air sacs in the pigeon is very similar to that of the fowl. The principal differences are indicated below.

Tertiary bronchi are much more numerous, and are absolutely and relatively smaller in diameter ($\frac{1}{2}$ mm.). Presumably this provides a greater number of air capillaries and hence a greater respiratory surface in the lung of a strong flying bird, than is necessary for the virtually flightless fowl.

Connexion between the anterior thoracic air sac and the lung is slightly different from the fowl. In the fowl, several tertiary bronchi connect directly with this sac.

Table 4. *A summary of air sac connexions to the lungs, extensions into bones and relations with other organs in the domestic fowl*

Sac	Paired or unpaired in adult	Relative sizes of air sacs compared with the posterior thoracic sac	Connexion to lung	Extensions		Relations with organs in the body cavity
				Into bones	To other areas	
Cervical	Unpaired	2	By direct termination of no. 1 anterior dorsal secondary bronchus from each lung	Cervical and thoracic vertebrae	None	Almost completely surrounded by the interclavicular sac. Oesophagus lies between it and the interclavicular sac. Extends back between the lungs to about their mid-point
Interclavicular	Unpaired	10 (including axillary)	(1) By a large duct from no. 3 anterior dorsal secondary bronchus. (A similar duct with the same origin leads to the anterior thoracic sac) (2) By several (6–9) tertiary bronchi which are connected to (i) No. 1 anterior dorsal secondary bronchus (1st ventral branch) (ii) No. 4 anterior dorsal secondary bronchus (lateral branch)	Humerus (via axillary sac). Coracoids. Sternum (mostly in rostrum not into keel)	Axillary sac which leads into the humerus	This is the most complicated sac of all. It surrounds the cervical sac, the lower part of the trachea, the syrinx and the first parts of each primary bronchus. The ventral part of the sac lies directly on top of the sternum, under the apex of the heart. It fills the entrance to the body cavity and lies between the two coracoids and the clavicles. The scapula and coracoid are in contact with the lateral aspect of the sac. The oesophagus lies immediately above it
Anterior thoracic	Paired	3	(1) By a large duct from no. 3 anterior dorsal secondary bronchus which originates in common with a similar duct leading to the interclavicular sac (2) By a group (6–10) of tertiary bronchi connected to (i) No. 1 anterior dorsal secondary bronchus (1st ventral branch) (ii) No. 4 anterior dorsal secondary bronchus (lateral branch)	None	None	They lie within the rib cage immediately below each lung. They enclose between them the heart, the anterior part of liver and proventriculus, and part of the oesophagus before it enters the proventriculus

Table 4 (*cont.*)

Sac	Paired or unpaired in adult	Relative sizes of air sacs compared with the posterior thoracic sac	Connexion to lung	Extensions: Into bones	Extensions: To other areas	Relations with organs in the body cavity
Posterior thoracic	Paired	1 (smallest sac of all)	By a group of tertiary bronchi around the posterior, ventrolateral corner of the lung, which enter a short common duct. This common duct (diam. 4 mm.) then enters the sac. By the direct termination of no. 2 posterior ventral secondary bronchus, which also enters the common duct. Very similar to posterior thoracic sac	None	None	Situated under the posterior part of the rib cage, between the body wall and the anterior, lateral part of the abdominal air sac
Abdominal	Paired	22 (largest sac of all). Left is a little larger than right	A group of tertiary bronchi, close to those entering the posterior thoracic sac, enters a short common duct which then joins the abdominal sac. By direct termination of the primary bronchus which, at this point, is of tertiary-bronchial size	Lumbar vertebrae	Round the anterior aspect of the hip joint. A large sheet-like extension above the kidneys, in close contact with the sacrum	The abdominal sacs are related to most of the abdominal viscera. The posterior limit of each is ventral to the cloaca and pygostyle Surface of sac — Related to Left sac: Dorsal — Left kidney Ventral — Spleen, proventriculus, gizzard Lateral — Body wall, left posterior thoracic sac Medial — Left testis, small intestine, caeca, colon Right sac: Dorsal — Right kidney Ventral — Small intestine Lateral — Body wall, right posterior thoracic sac Medial — Right testis, small intestine, caeca, colon

In the pigeon, these tertiary bronchi (which are connected to much the same part of the lung as in the fowl) unite to form a single large duct which then joins the anterior thoracic sac, in common with the other large duct connecting the sac to no. 3 anterior dorsal secondary bronchus (Pl. 5). At the termination of no. 1 anterior dorsal secondary bronchus in the cervical sac of the pigeon, the duct has a greater diameter (both relatively and absolutely) than its counterpart in the fowl.

In the posterior group of secondary bronchi, the ventral series of ducts, although present, is not so well developed as in the fowl, and the last two or three may be indistinguishable from tertiary bronchi. The first two or three, however, are clear and the second connects the primary bronchus directly to the posterior thoracic sac, as it does in the fowl. This is the duct which is frequently misinterpreted as a bifurcation of the primary bronchus.

There are two unfused cervical air sacs in the pigeon, each of which is connected to the pneumatic cervical vertebrae. The posterior thoracic air sac has a different shape from that in the fowl and lies more between the lung and the abdominal sac. It is also relatively larger in the pigeon. The sternum is pneumatic to a much greater extent in the pigeon than in the fowl. A very large extension from the interclavicular sac is present in the pigeon between the pectoral muscles, and might be called the pectoral sac. This is either absent in the fowl or very much less well developed. The medial surface of the primary bronchus in the pigeon is not entirely free from connexions to subsidiary bronchi, but is connected to several tertiary bronchi over its posterior half.

THE STRUCTURE OF THE RESPIRATORY SYSTEM IN THE DUCK (*ANAS PLATYRHYNCHA*)

The general pattern of the lungs and air sacs in the duck is the same as that of the fowl and pigeon. There are, however, a few minor differences which are indicated below. Perhaps the most striking feature of the duck's lung is the fact that the posterior dorsal secondary bronchi emerge on to the lateral side of the lung and show very clearly how the tertiary-bronchial arcades link together the posterior and anterior groups of secondary bronchi (Pl. 6).

In the fowl and pigeon the posterior dorsal secondary bronchi are not visible at all from the lateral side, but are screened from it by a layer of tertiary bronchi. Also on the lateral side of the duck's lung a fairly large duct (diam. 2 mm.) is visible which is connected directly to the abdominal air sac at the same point as the termination of the primary bronchus. This duct is connected to many tertiary bronchi on its medial, dorsal and ventral surfaces and is absent in the fowl and pigeon.

The course of the primary bronchus through the lung and its connexion to an anterior and a posterior group of secondary bronchi is much the same as in the fowl and pigeon. In all three species the two groups of secondary bronchi are separated by a short length of primary bronchus which is completely devoid of subsidiary bronchial connexions. The casts showed no sign of a 'vestibule' in the primary bronchus, although they did show that many tertiary bronchi are connected to the medial surface of its posterior half (in the fowl the medial surface of the primary bronchus is free from subsidiary bronchial connexions over its entire length). They also showed that the primary bronchus in the duck is a good deal larger than a tertiary bronchus at its point of termination in the abdominal sac.

The four anterior dorsal secondary bronchi have much the same arrangement in the duck as in the fowl and pigeon, except that no. 2 anterior dorsal secondary bronchus sends a large branch round the lateral side of the primary bronchus to the ventral part of the lung where it is connected to many tertiary bronchi. (This branch is absent in the fowl and pigeon.) Seven posterior dorsal secondary bronchi are connected to the primary bronchus as in the fowl and pigeon, although their appearance on the lateral side of the lung is peculiar to the duck. The first duct of this posterior dorsal series has a large ventral branch which runs parallel to the first posterior ventral secondary bronchus, and is connected to tertiary bronchi in the ventral part of the lung. (No such large ventral branch is present in the lung of either the fowl or pigeon.)

Three posterior ventral secondary bronchi are well developed and are similar to those in the pigeon. The second of these three ducts is connected directly to the posterior thoracic air sac, as well as to many tertiary bronchi, and is frequently misrepresented as a bifurcation of the primary bronchus.

The basic distribution of the air sacs is much the same as in the fowl and pigeon although there are a few minor differences. The cervical sac is paired in the duck and pigeon but single in the fowl. The posterior thoracic sac in the duck is almost as large as the abdominal sac. This is associated with a relatively larger no. 2 posterior ventral secondary bronchus than is found in the fowl, where the posterior thoracic sac is very much smaller than the abdominal sac (Table 4).

Connexions between the lungs and air sacs are basically the same in the three species. The anterior thoracic sac in the fowl is connected to the lung by a large duct (from no. 3 A.D.S.B.) and also by several (6–8) tertiary bronchi which enter the sac independently. In the pigeon these tertiary bronchi are connected to a large distinctive duct which enters the anterior thoracic sac in common with the large duct from no. 3 anterior dorsal secondary bronchus (Pl. 5). In the duck, the arrangement is the same as it is in the fowl except that very many more tertiary bronchi are connected directly to the sac.

However, in spite of these various minor differences between the duck and pigeon on the one hand and the fowl on the other, only one is likely to be of any real functional significance and this concerns the relative sizes of their tertiary bronchi. The duck and pigeon are both strong flying birds and have at least four times as many tertiary bronchi per unit volume of lung as are present in the relatively flightless fowl.

DISCUSSION

The present observations are based on a study of thirty-eight casts (20 fowl, 12 pigeon, 6 duck), which have shown, within each species, a remarkable consistency of structural detail. This suggests that the technique is reliable and that the casts are accurate. However, it is still important to consider whether any distortion results from the pressure of injection and, if it does, to what extent this is significant from the point of view of the anatomical study for which the casts were intended. In fact there does not appear to be any significant distortion anywhere in the system.

This can be confirmed by making direct measurements of tertiary bronchi in a fresh lung and comparing their diameters with others taken from a cast. In both cases the diameters are virtually the same (fowl 1 mm., pigeon $\frac{1}{2}$ mm., duck $\frac{1}{2}$ mm.).

It would appear that the whole system is so tightly packed into the body cavity that over-distension of one part is prevented by the simultaneous filling of an adjacent part. Similarly, in the lungs, tertiary bronchi are themselves so tightly packed that no significant distortion occurs. The air sacs are undoubtedly distended to their maximum size and the most likely place for distortion is in the large abdominal sacs which are not so completely surrounded by relatively indistensible structures, as are all the others. However, even here distortion is small and in no way upsets the nature of their connexions to the lungs.

One of the most widely published illustrations of the avian lung today is that taken from a paper by Brandes written in 1924. This illustration refers to the pigeon and presents a rather different picture of its lung structure from that which has resulted from a study of the present series of latex casts. The principal differences are summarized below.

None of the present series of casts showed any sign of a large swelling ('vestibule') in the anterior part of the primary bronchus. In every case the primary bronchus in the pigeon maintained a uniform diameter of about 3 mm. over its anterior half and then progressively reduced its diameter until it was about the same size as a tertiary bronchus ($\frac{1}{2}$ mm. diam.) at the point where it joined the abdominal air sac. Brandes showed the primary bronchus at this point of connexion to be about six times as large as was the case in any of the present casts.

Brandes's 'ventrobronchi' and 'dorsobronchi' correspond respectively to the anterior dorsal secondary bronchi and to the dorsal series of posterior secondary bronchi of the present work. He makes no reference to the ventral series of posterior secondary bronchi, although the second duct of this series is misrepresented as a bifurcation of the primary bronchus. Three large 'ventrobronchi' are shown connected to the 'vestibule'. These are called 'ventrobronchus cervicalis' (connected to the cervical and interclavicular sacs), 'ventrobronchus medialis' (not connected to any sac) and 'ventrobronchus caudalis' (connected to the anterior thoracic sac). A large branch from the 'ventrobronchus caudalis' is called 'ventrobronchus lateralis'.

Latex casts used in the present study show that four large ducts are connected to the dorsal surface of the primary bronchus immediately after it enters the lung. In this paper they have been called anterior dorsal secondary bronchi numbers 1, 2, 3 and 4. Clearly they correspond to Brandes's 'ventrobronchi' but their direct connexions to the air sacs are significantly different. No. 1 anterior dorsal secondary bronchus is connected only to the cervical sac. No. 2 is not connected to any sac. No. 3 is connected both to the interclavicular and anterior thoracic sacs. No. 4 is not connected to any sac.

In Brandes's illustrations the cervical sacs are shown with a posterior extension running back between the lungs and extending posteriorly beyond them. This extension does not exist. On the other hand he makes no reference to a large duct which connects several tertiary bronchi on the antero-ventral aspect of the pigeon's lung to the anterior thoracic sac (Pl. 5). This is different from the arrangement in the fowl where these tertiary bronchi connect independently to the anterior thoracic sac. Brandes's account was based on the assumption that valves existed at the junction of all ducts with air sacs. These valves have never been found.

In the fowl about twenty small ducts are connected to the lateral surface of the primary bronchus (Table 1) and have been described as 'lateral secondary bronchi' by King (1956). However, they appear to be identical with tertiary bronchi in other parts of the lung and there would seem to be little reason for calling them anything else. These tertiary bronchi connect the lateral wall of the primary bronchus anteriorly to the ventral branches of no. 1 anterior dorsal secondary bronchus, and posteriorly to the posterior thoracic and abdominal air sacs.

In most accounts of the avian respiratory system importance has been attached to the so-called 'recurrent bronchi'. These have been described as ducts which develop from the air sacs and grow back into the lungs where they anastomose with tertiary bronchi, thus providing a special return route from the sacs to the lungs. In other words, air is considered to enter a sac by one duct or set of ducts, and to leave by another. Whilst there would appear to be convincing evidence for the development of these 'recurrent bronchi' in the embryo (Locy & Larsell, 1916), they are indistinguishable from tertiary bronchi in the definitive lung and most probably correspond to the tertiary bronchi referred to above, which connect the posterior thoracic and abdominal air sacs to the lateral wall of the primary bronchus.

Latex casts used in the present study show that five of the eight air sacs (abdominals, posterior thoracics and cervical) in the fowl, six of the nine in the duck (where the cervical sac is paired) and eight of the nine in the pigeon are connected to tertiary bronchi of the lungs only by a single duct (Text-figs. 1, 2). In the fowl and duck the remaining three sacs (interclavicular and paired anterior thoracics), and the remaining one sac (interclavicular) in the pigeon, are linked to the lungs by more than one duct, but in the absence of evidence for valves anywhere in the system it is difficult to see how 'recurrent bronchi' could function even in these sacs. In the pigeon, where many tertiary bronchi from the ventral part of the lung are connected to a large and distinctive duct (Pl. 5), which then connects with the large duct from no. 3 anterior dorsal secondary bronchus, so that they both join the anterior thoracic sac together, there is no possibility whatever of 'recurrent bronchi'. It is suggested that this term, which was originally introduced by Schulze in 1910, may be misleading.

Perhaps it is worth emphasizing two points. First, that two pairs of air sacs have almost identical connexions to the lungs. These are the abdominal and posterior thoracic sacs on the one hand, and the anterior thoracic and interclavicular sacs on the other. Secondly, that there is a short distance along the primary bronchus, between the anterior and posterior groups of secondary bronchi, which is completely free from connexions to any subsidiary ducts.

In this account of the comparative anatomy of the respiratory pathways in the domestic fowl, pigeon and duck, care has been taken to avoid functional implications. However, it is hoped that subsequent cineradiographic work, which will be based on the above description, may contribute to our understanding of the *modus operandi* of the most complicated respiratory system that has ever evolved.

SUMMARY

1. A description of the respiratory pathways in the domestic fowl, pigeon and duck is presented, together with the main structural differences between the respiratory systems of these species.

2. It is suggested that in all three species there are only two groups of secondary bronchi, which should be called anterior and posterior, and not four; that the anterior group is dorsal and not ventral or ventromedial; and that the posterior group consists of both dorsal and ventral ducts, but that there is no lateral group of secondary bronchi. The so-called 'lateral secondary bronchi' are really tertiary bronchi which connect the posterior lateral wall of the primary bronchus to the ventral branches of no. 1 anterior dorsal secondary bronchus, and also to the posterior thoracic and abdominal air sacs.

3. In the fowl each of the four anterior dorsal secondary bronchi is connected at right angles to the dorsal surface of the primary bronchus by a narrow neck (diameter about 2 mm.), beyond which they are dilated to become the largest ducts in the lung. Each of the sixteen posterior secondary bronchi has a very slight constriction just before it joins the primary bronchus, but for the most part these ducts maintain a uniform diameter of about 3 mm. They meet the dorsal and ventral surfaces of the primary bronchus at an angle of about 60°. The arrangement in the pigeon and duck is very similar.

4. In the fowl the primary bronchus is free from connexions to subsidiary ducts on all sides for a short distance between the two groups of secondary bronchi, and along the entire length of its medial surface. It describes a flattened horizontal 'S' bend in its route through the lung and terminates in the abdominal air sac, where it is about the same size as a tertiary bronchus. There is a similar arrangement in the pigeon and duck. In all three species direct connexion between the primary bronchus and the posterior thoracic air sac is by the second posterior ventral secondary bronchus, and not by a bifurcation of the primary bronchus. There was no sign of a so-called 'vestibule' in the anterior part of the primary bronchus.

5. Tertiary-bronchial arcades link together the two groups of secondary bronchi and make up the bulk of each lung in all three species.

6. Two pairs of air sacs (abdominal and posterior thoracic; anterior thoracic and interclavicular) have almost identical connexions to the lung in all three species.

7. Each side of the cervical air sac in the fowl, and each separate cervical sac in the pigeon and duck, is connected to the lung by a single duct. In the abdominal and posterior thoracic sacs of the three species, and the anterior thoracic sac of the pigeon, a group of several tertiary bronchi enter a common duct which then joins the air sac. In the fowl and duck the two anterior thoracic and the interclavicular sacs, and in the pigeon the interclavicular, are connected by more than a single duct.

The so-called 'recurrent bronchi' in the definitive lung of all three species are indistinguishable from tertiary bronchi which link the posterior thoracic and abdominal air sacs to the lateral wall of the primary bronchus. In the absence of valves the whole concept of 'recurrent bronchi' in the adult is of doubtful value.

8. The pigeon and duck, both strong flying birds, have at least four times as many tertiary bronchi per unit volume of lung as has the relatively flightless fowl.

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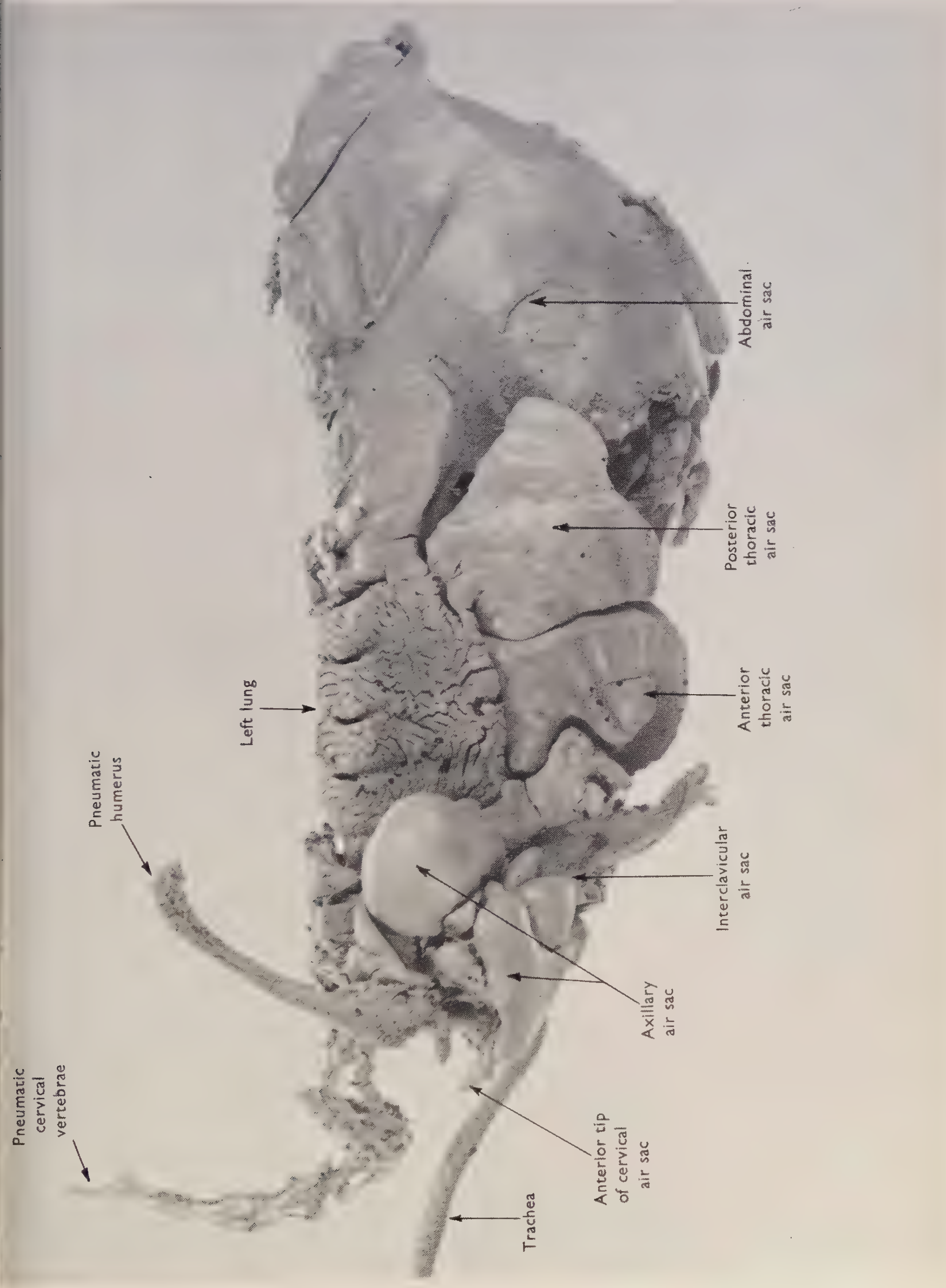
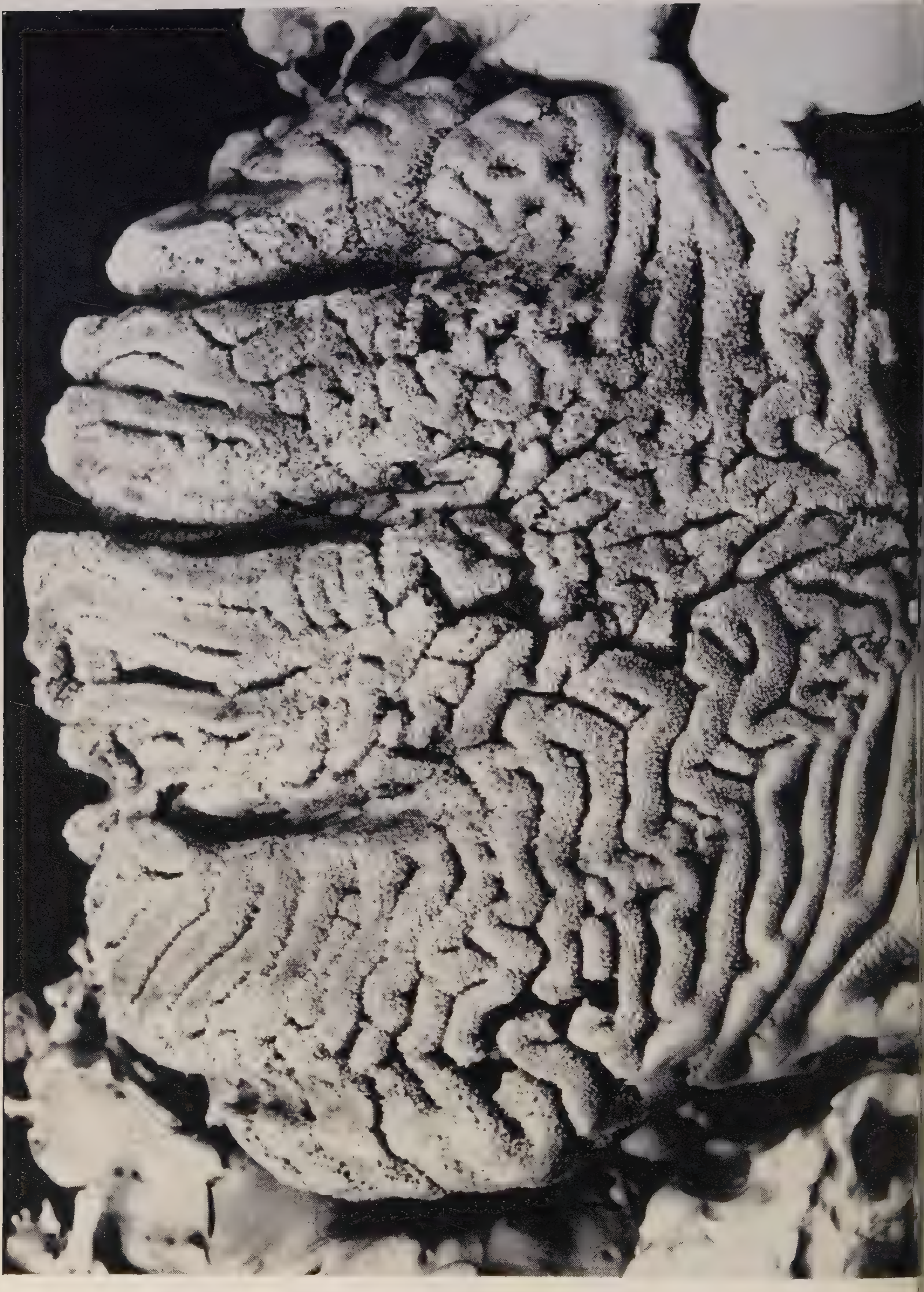
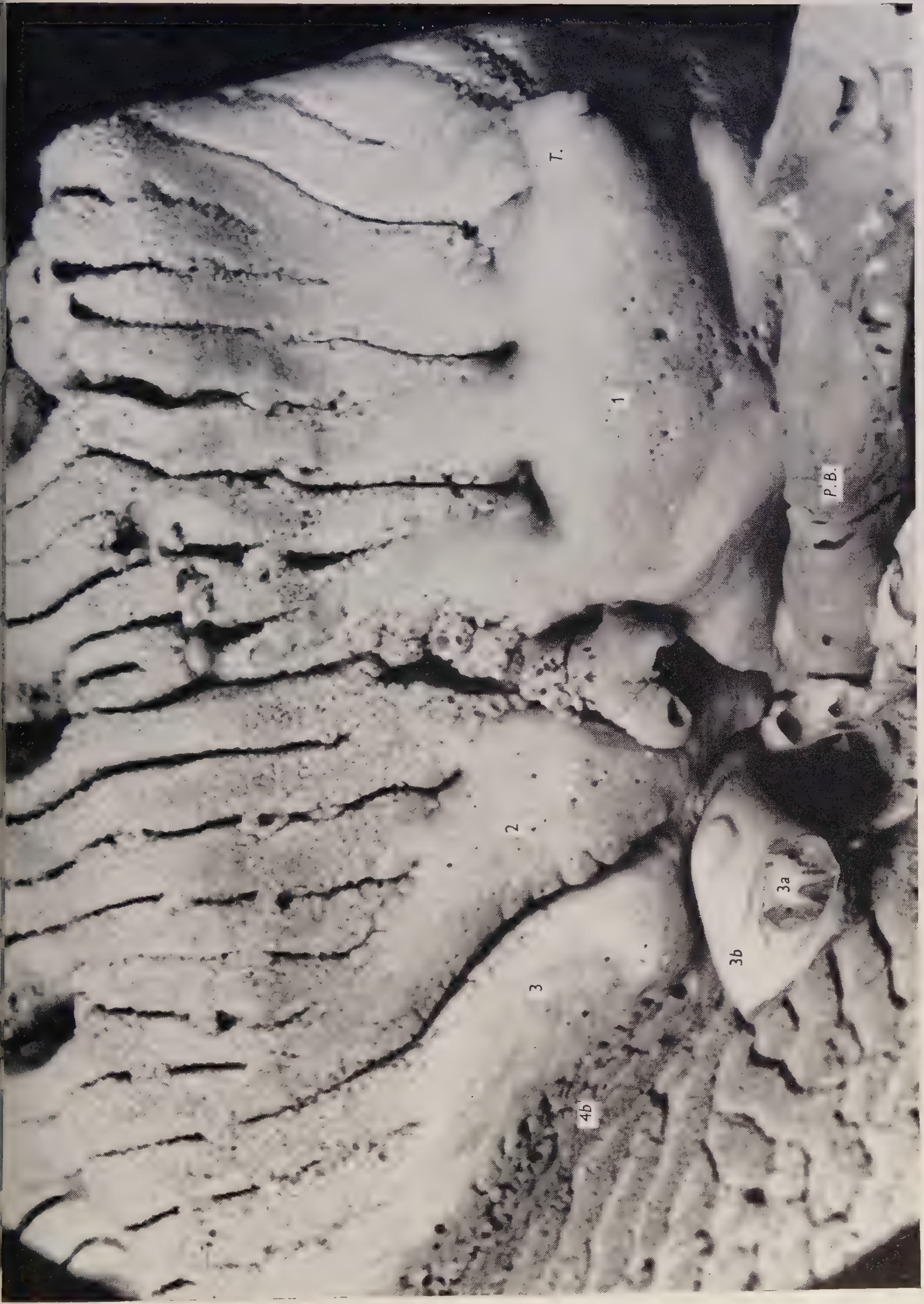

Abdominal air sac
Posterior thoracic air sac
Anterior thoracic air sac
Left lung
Interclavicular air sac
Pneumatic humerus
Axillary air sac
Pneumatic cervical vertebrae
Anterior tip of cervical air sac
Trachea

AKESTER—Comparative anatomy of the respiratory pathways



AKESTER—Comparative anatomy of the respiratory pathways



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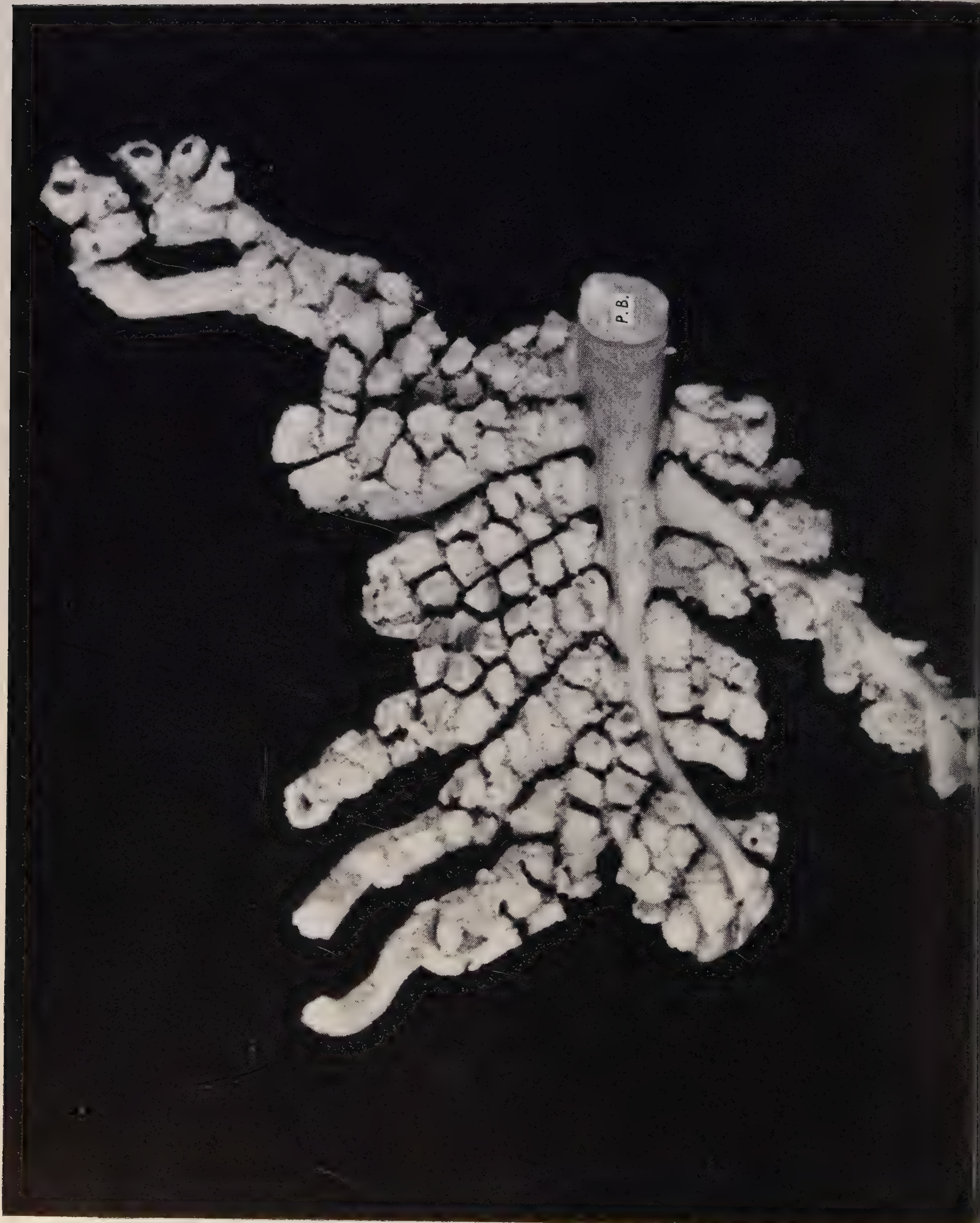

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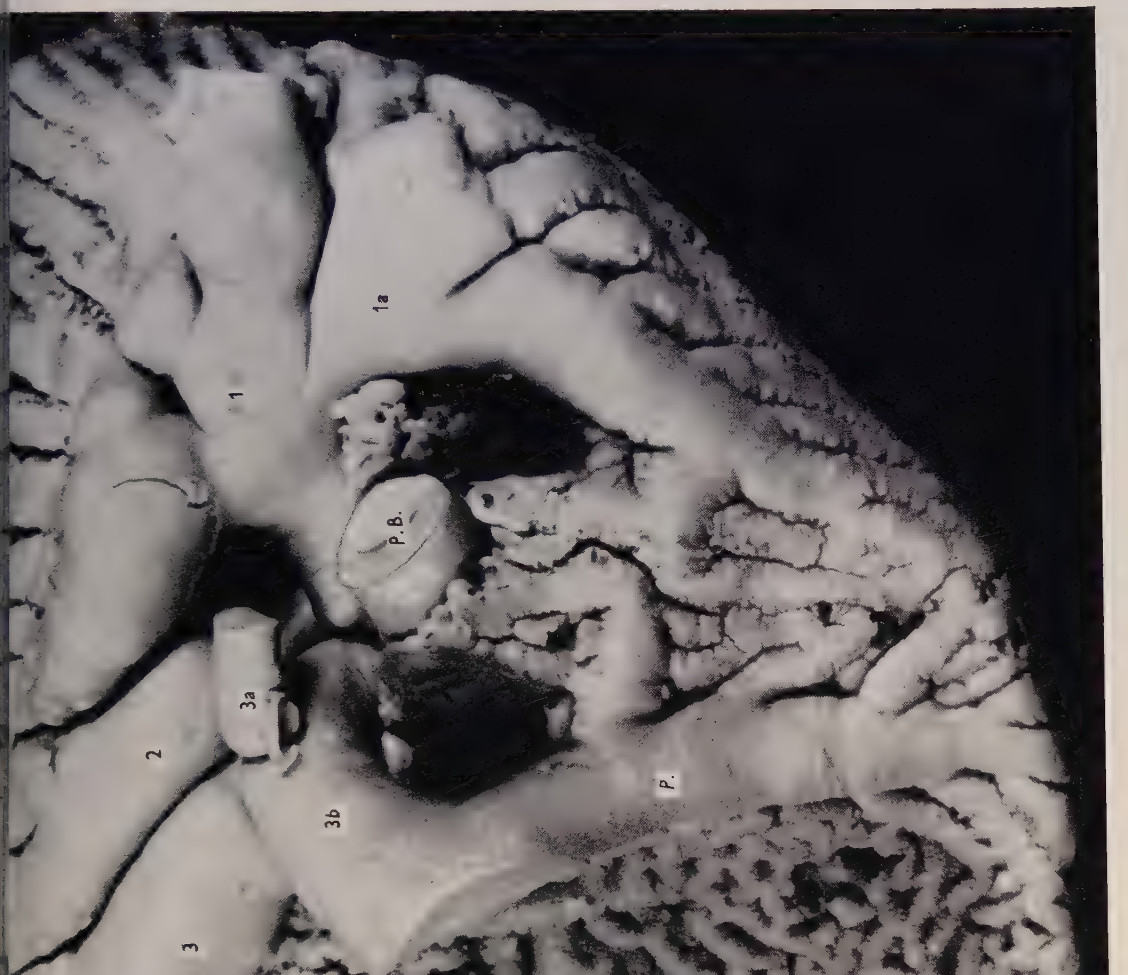

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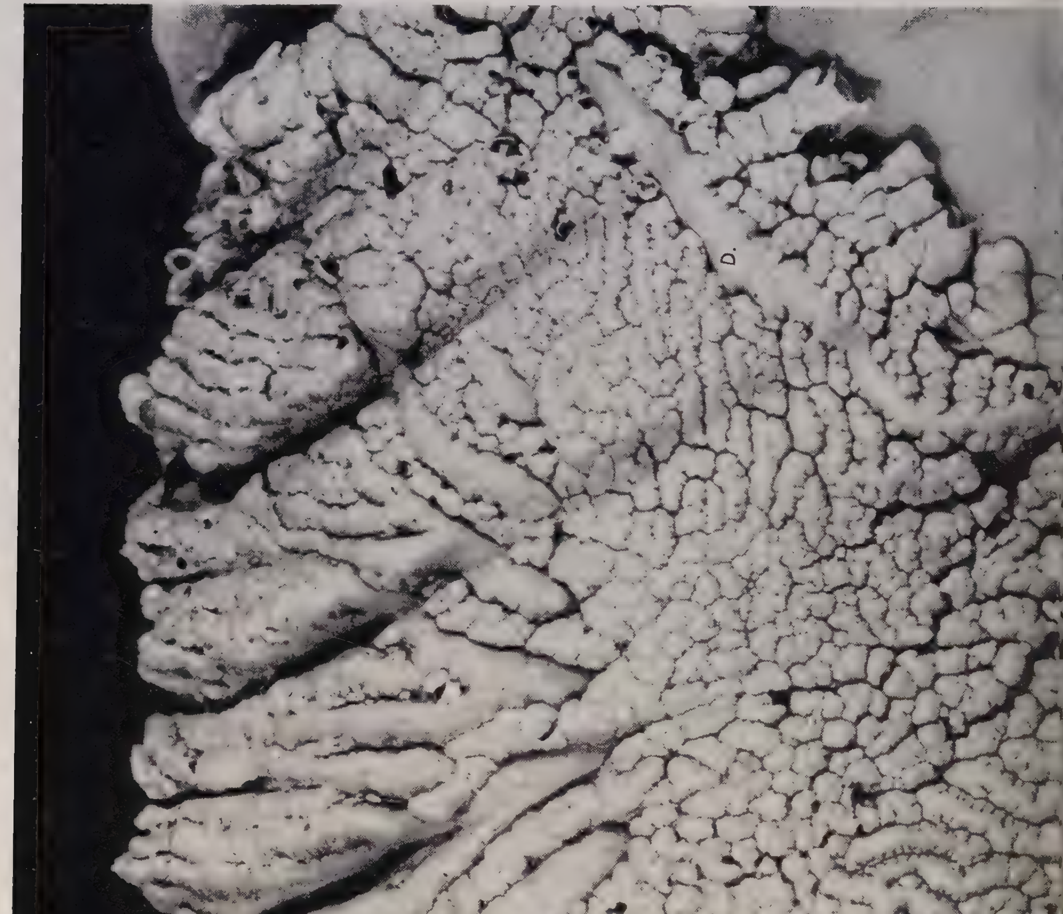

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KEY FOR ALL ILLUSTRATIONS

1	No. 1 anterior dorsal secondary bronchus.
1*a*	First ventral branch of no. 1 anterior dorsal secondary bronchus.
2	No. 2 anterior dorsal secondary bronchus.
3	No. 3 anterior dorsal secondary bronchus.
3′	Large duct from no. 3 anterior dorsal secondary bronchus which bifurcates into 3*a* and 3*b*.
3*a*	Large duct leading directly to the interclavicular air sac.
3*b*	Large duct leading directly to the anterior thoracic air sac.
4	No. 4 anterior dorsal secondary bronchus.
4*a*	Lateral branch of no. 4 anterior dorsal secondary bronchus.
4*b*	Posterior branch of no. 4 anterior dorsal secondary bronchus.
P.	Large duct on the medial side of the pigeon's lung which connects tertiary bronchi in the ventral part of the lung to the anterior thoracic sac.
D.	Large duct on the lateral side of the duck's lung which connects the abdominal sac to tertiary bronchi on the lateral aspect of the lung.
P.B.	Primary bronchus.
T.	Termination of no. 1 anterior dorsal secondary bronchus in the cervical air sac.

EXPLANATION OF PLATES

Plate 1. Whole cast showing lung and air sacs of the fowl (left lateral view).

Plate 2. Left lung of the fowl (lateral view). Tertiary bronchi can be seen ramifying over the surface of the lung.

Plate 3. Left lung of the fowl (medial view). To illustrate the four anterior dorsal secondary bronchi. These secondary bronchi are all connected to the dorsal surface of the primary bronchus. Connexions of the first two can be seen in the illustration. No. 1 anterior dorsal secondary bronchus connects with many tertiary bronchi and finally terminates in the cervical air sac.

Plate 4. The posterior group of secondary bronchi in the lung of the fowl (medial view). The primary bronchus has been cut in half. In this case the second posterior ventral secondary bronchus connects directly with the posterior thoracic air sac. It thus corresponds to the arrangement shown in the left lung of Text-fig. 4. Note that posterior secondary bronchi connect with the primary bronchus at a slight angle, on both its dorsal and ventral surfaces, but that no ducts connect with its medial surface.

Plate 5. Left lung of the pigeon (medial view). Note the large duct (*P.*) which connects tertiary bronchi on the ventral aspect of the lung to the anterior thoracic sac. The anterior thoracic sac itself has been removed.

Plate 6. Left lung of the duck (lateral view). Note how the posterior dorsal secondary bronchi penetrate the tertiary bronchi and are visible from the lateral side (cf. Pl. 2). The large duct (*D.*) which connects the abdominal sac with many tertiary bronchi is also shown.

AUDITORY CORTEX OF THE SEAL (*PHOCA VITULINA*)

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Descriptions of the brain of the seal deal mainly with its external appearance. The few maps of functional localizations which have been made have indicated the site of the motor cortex. Rioch (1937) and Langworthy, Hesser & Kolb (1938) obtained movements of different parts of the body on electrical stimulation of the region of cortex lying behind the orbit in front of the cruciate fissure. There are no reports of experimental mappings of cortical sensory areas in these animals. In this short report, the site of responses to click stimulation of the ear and to tactile stimulation of the face in the common seal (*Phoca vitulina*) will be described.

METHOD

Three unweaned seal pups of between 8 and 12 kg. body weight were anaesthetized in the laboratory with a solution of thiopentone Na (Pentothal) injected into the internal vertebral venous plexus (Harrison & Tomlinson, 1956) of the lumbar region through a needle inserted between the lateral parts of neighbouring vertebral laminae. Sufficient solution was injected slowly until the seal lost consciousness, about 150–200 mg. of thiopentone being needed. At this point the animals stopped breathing; after quick tracheotomy and insertion of a cannula, ventilation was maintained by intermittent positive pressure ventilation with room air or with 100% oxygen if the arteries appeared blue. Anaesthesia was deepened and maintained by small quantities of pentobarbitone Na (Nembutal) delivered through a cannula inserted into a suitable forelimb vein, the additions of anaesthetic being made at intervals to maintain the slope of a graph of the increments plotted against time. After reflecting the overlying skin and removing the temporalis muscle, wide craniotomy exposed the superolateral surface of the left cerebral hemisphere. The *dura mater* was cut and reflected under a pool of warm paraffin oil. During the experiment the rectal temperature was held steady by pouring cold water over the animal's body at intervals.

The exposed cortex was explored in 2 or 4 mm. co-ordinates with a ball-ended Ag wire electrode, using another Ag wire inserted into scalp muscle as indifferent electrode. The cortical evoked potentials were visualized and photographed on an oscilloscope after r.c.-coupled amplification. The click stimulus was produced by a brief square-pulse fed into a loud speaker near the head, and tactile stimulation of the face was done by tapping the mystacial pad of the right upper lip with a rod mounted on a solenoid. Both click and tap were synchronized with the oscilloscope time-base. The evoked potentials were recorded point-by-point off the cortex, and subsequently the photographs of the responses were mounted on charts of the cortical landmarks constructed on the same co-ordinates. At death the body of the seal was perfused with 10% formalin saline and the hardened brain was removed, cleaned of its membranes and photographed.

RESULTS

Anaesthesia was maintained at such a depth that the evoked cortical potentials consisted of positive waves with little or no subsequent negative activity. Click-evoked potentials were recorded within an area lying behind the vertical limb of the pseudo-sylvian fissure and forming a part of the surface of the posterior sylvian gyrus (Fig. 1). The recorded activity did not spread further in front of the fissure than might be accounted for by spread of electrical pick-up, and the area of posterior Sylvian gyrus lying between the active area and the supra-Sylvian sulcus above was also clear of activity. The potentials were shown to be of auditory origin by their disappearance when masked by a continuous loud noise.

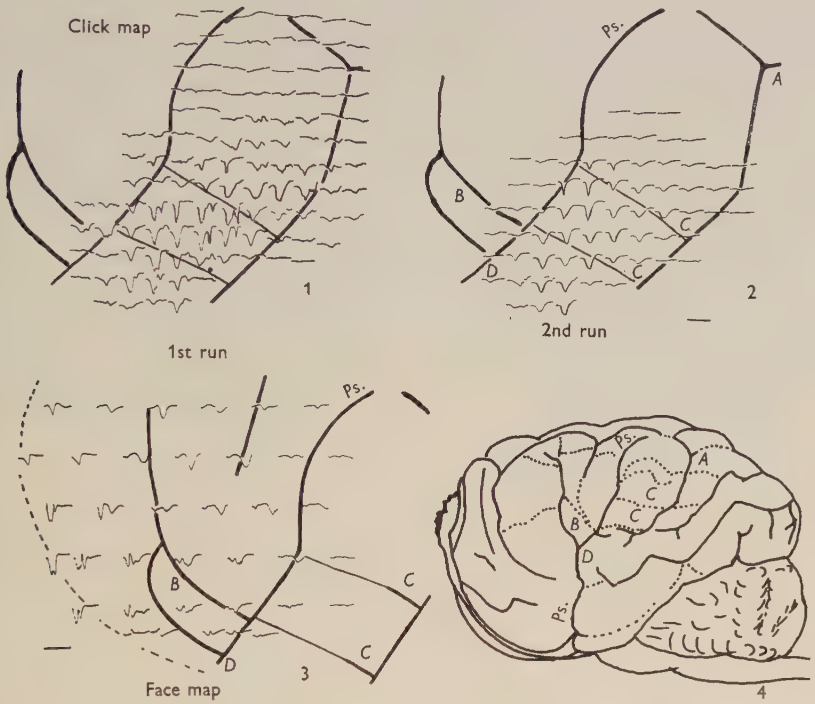

Fig. 1. Evoked-potential maps of auditory and face areas in a seal. Thiopentone-pentobarbitone anaesthesia. (1 and 2) Click maps: 1st run was done under lighter anaesthesia than was 2nd run done later, both mappings being on 2 mm. co-ordinates. (3) Face map: made after click maps completed, on 4 mm. co-ordinates. Time maker 100 cycles. (4) Side view of the hardened brain cleaned of its membranes; dotted lines indicate grooves made by blood vessels. *A*, *B*, *C*, *D*: vascular grooves and sulcus intersections used in making outline chart of the explored area of the living brain and used to confirm locality on the isolated fixed brain. *Ps*: pseudo-Sylvian fissure.

The face area, as defined by tapping the contralateral upper lip on the whisker-bearing mystacial pad, extended back towards the vertical pseudo-sylvian fissure (Fig. 1). The anterior limit of the face area was not defined because insufficient cortex was exposed. These responses were not masked by noise. No activity was set up in the exposed cortical area by tapping the limbs or body.

When the various mappings were related to the furrows of the brains, the lie of the auditory (click) and part of the face (upper lip) areas could be related to the pseudo-Sylvian and supra-Sylvian furrows. Fig. 2 summarizes the findings, the mapped areas being placed on a drawing made from a photograph of a hardened brain stripped of its membranes. The motor area, previously described by Rioch (1937) and Langworthy *et al.* (1938), has also been included.

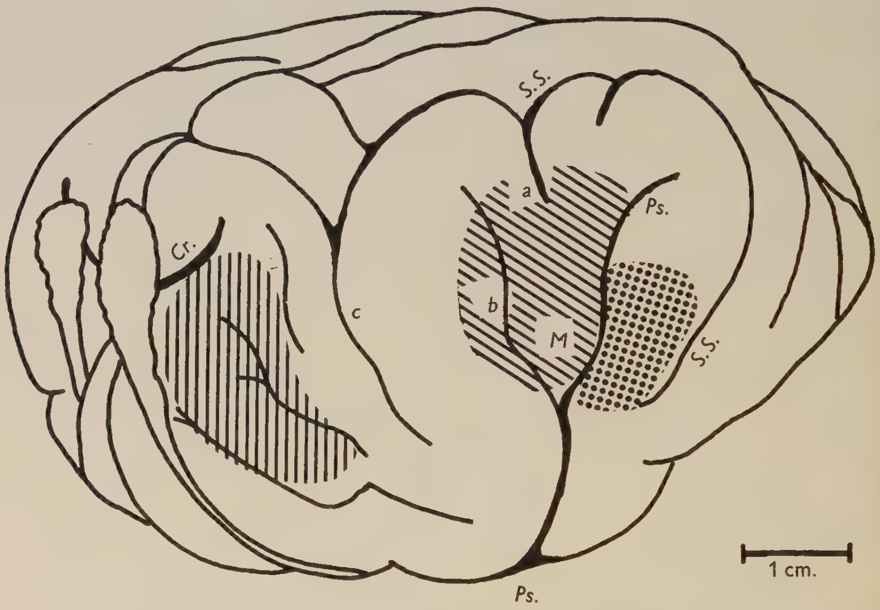

Fig. 2. Drawing, traced from a photograph, of the brain of a seal after hardening and removal of vascular membranes. Vertical hatching = motor area of Rioch (1937) and Langworthy *et al.* (1938). Dotted = auditory (click) response area. Diagonal hatching = part of face area (tapping contralateral upper lip on mystacial pad).

S.S. supra-Sylvian sulcus. *Ps.* pseudo-Sylvian fissure, with vertical posterior limb named Sylvian fissure by Fish (1898). *Cr.* cruciate sulcus. *a*, anterior end of supra-Sylvian sulcus according to Fish. *b*, 'presuper-Sylvian' sulcus of Fish. *c*, anterior supra-Sylvian sulcus according to Rioch and Langworthy *et al.*, ansate-coronal sulcus homologue according to Fish. *M*, gyrus of which ventral half is hidden in depth of the pseudo-Sylvian fissure.

DISCUSSION

The auditory response area was more limited than had been expected from experience with another carnivore, i.e. cat (Downman, Woolsey & Lende, 1960). In the cat the area which can be activated by direct electrical stimulation of exposed cochlear nerve fibres fills most of the surface bounded by the supra-Sylvian sulcus, and click stimulation produces evoked potentials in much of it. Accepting the usual naming of the so-called supra-Sylvian sulcus in the seal (Rioch, 1937; Langworthy *et al.* 1938) as a topographical convenience, it is seen that only a limited part of the cortex within its boundary was activated. It must be emphasized that the animals were young, being about 8–12 days old. It is possible that the restriction of the active area represents a degree of immaturity in the auditory projection system, although it is notable that impulses from the snout could activate other areas within the supra-Sylvian sulcus boundary. It is probable that much of the auditory cortex is hidden within the folds of the pseudo-Sylvian fissure. On separating the lips of the fissure it is seen to be deep and contains a gyrus of which the dorsal half appears at the surface (*M* in Fig. 2), while the ventral half is concealed by the overhanging lips of the fissure. As Fish (1898) has described, the two arms of the forked pseudo-Sylvian fissure are two separate furrows, which meet about half way down; it is the stem and posterior arm of this fissure which was named as the Sylvian fissure in Fish's description. The anterior arm of the Y (presuper-Sylvian sulcus of Fish; *b*, in Fig. 2) extends down into the anterior wall of the fissure to separate two gyri forming part of that wall. It is the more medial of these gyri that appears on the lateral surface of the brain (gyrus *M*. in Fig. 2), the lateral gyrus forming the anterior lip of the lower part of the pseudo-Sylvian fissure. Fish designated the sulcus labelled *a* in Fig. 2 as being the forward end of the supra-Sylvian sulcus. What has been called

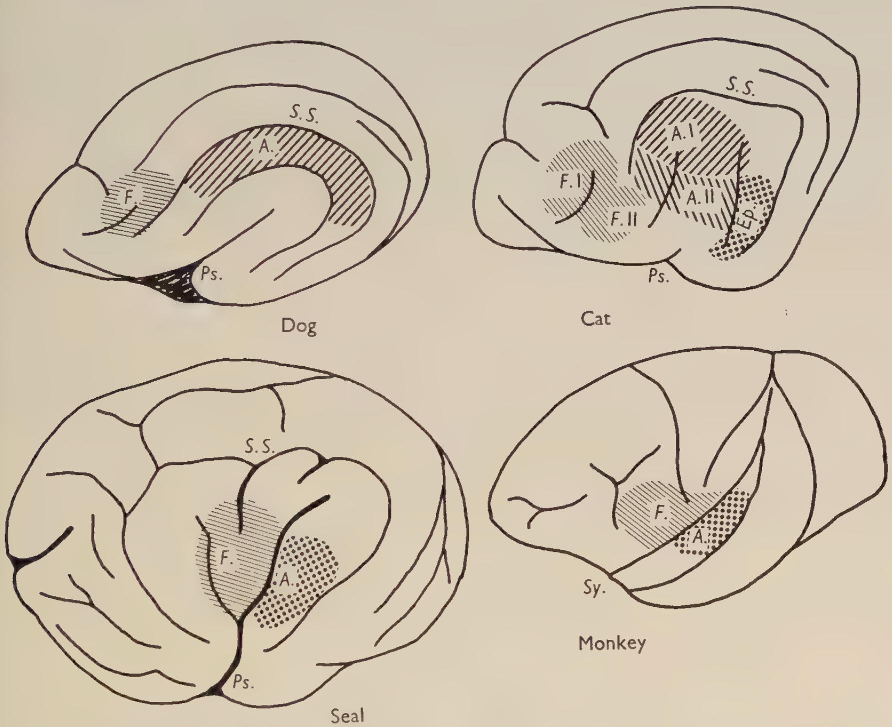

Fig. 3. Diagrams comparing the siting of auditory and face sensory areas as mapped in various animals. Dog: auditory area, A. (Tunturi, 1950); face, *F.* (Adrian, 1941). Cat: auditory areas *A.*I, *A.*II and *Ep.* (Downman *et al.* 1960); face areas, *F.*I and F.II (Adrian, 1941; Woolsey & Fairman, 1946). Monkey: auditory, *A.*; face, *F.* (Woolsey & Fairman, 1946, *macaque*; Hirsch & Coxe, 1958, *cebus*). Seal: this investigation. *S.S.* = supra-Sylvian sulcus. *Ps.* = pseudo-Sylvian fissure. *Sy.* = Sylvian fissure. Diagram not to scale of brain size.

elsewhere the anterior part of the supra-Sylvian sulcus is considered by Fish to represent the coronal and ansate sulci of other brains. Such an homology would fit in better with the present mappings, so far as they go, without having to assume that sensory areas in the brain of *Phoca* have shifted sideways relative to the furrowing pattern.

Comparing the mapped areas of the seal brain with published diagrams showing auditory and face areas of other carnivores and of primates (Fig. 3), it is seen that where the brain is less folded, as in the cat, the areas are in relation across the anterior limb of the supra-Sylvian sulcus. Where there has been much folding to produce the Sylvian fissure there is produced a situation which is mimicked in the seal; that is, a face area adjoining the auditory area across a deep furrow. Full identification of this face area, whether it is face I or face II (Woolsey & Fairman, 1946) will give a basis for better comparison with other brains and will give more evidence of the homologies of the foldings in this lateral area. The folding envisaged by Elliot Smith (1902, 1910) to lead to the formation of the Sylvian fissure would have included the anterior supra-Sylvian sulcus into the fissure, which would, from cat brain mappings, leave face I at the surface with face II in the depth of the fissure. This is the situation in the monkey (see Woolsey & Fairman, 1946). Carrying the comparison further, the part of the auditory cortex that lies out on the lateral surface of the brain in the superior temporal gyrus of primates, and also appears at the surface in the seal, might well be equivalent to the *Ep*. area of the cat auditory area (Rose & Woolsey, 1949; Downman *et al.* 1960).

SUMMARY

1. In seal pups of 8–12 days of age under barbiturate anaesthesia auditory (click) and face (tapping contralateral upper lip) stimulation caused evoked potentials at the exposed cerebral cortex.

2. The auditory response area lay within a part of the posterior Sylvian gyrus, being bounded anteriorly by the vertical limb of the pseudo-Sylvian fissure.

3. Face sensory area extended on to the anterior Sylvian gyrus, within the area enclosed by the so-called supra-Sylvian sulcus.

4. The large area of cortex lying within the folds of the pseudo-Sylvian fissure of the seal is emphasized.

The seal pups were presented to us by Prof. R. J. Harrison of the Department of Anatomy, the London Hospital Medical College, and for this we are most grateful. We express thanks also to Dr J. D. W. Tomlinson, of the same department, for instruction in the method of anaesthesia used. Helpful advice concerning brain anatomy was given by Prof. F. Goldby, of St Mary's Hospital Medical School, and by Professors R. E. M. Bowden and M. F. Lucas-Keene of this School, to whom thanks are also given.

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OBSERVATIONS ON THE NERVOUS SYSTEM OF THE LESSER BUSH BABY (*GALAGO SENEGALENSIS SENEGALENSIS*)

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The primates are classified into two main groups, the Strepsirhini and the Haplorhini, depending on the presence or otherwise of a naked rhinarium (Pocock, 1918; Hill, 1953). The area of naked rhinarium is well marked in some of the Strepsirhini such as true lemurs (suborder Lemuroidea) while it is greatly reduced in the Galagos (suborder Lorisoidea). The latter appear to link the Strepsirhini to *Tarsius*, the only living representative of Tarsoidea, now usually grouped with the Haplorhini although it was previously included in the Strepsirhini. Furthermore, Galagos not only show a superficial resemblance to *Tarsius* in their external appearance but also have a similar saltatory mode of progression and nocturnal habits. Besides, both these primates exhibit a striking similarity in a number of features pertaining to the skull. It is, therefore, of interest to inquire whether these similarities between *Galago* and *Tarsius* are, in fact, a reflexion of true affinity or merely an expression of parallel evolution.

Little is known of the anatomy of the *Galago* (family, Galagidae), although Murie & Mivart (1872), Hill (1932, 1933, 1936, 1953), Le Gros Clark (1934), Davies (1947) and others have contributed to our knowledge of some of the other representatives of the Strepsirhini. The brain of some of the Lemuroidea was investigated by Elliot Smith (1903) and that of *Microcebus murinus* by Le Gros Clark (1931). Among the Lorisoidea, the fissural pattern of the brain of *Nycticebus coucang* was described by Connolly (1950). Diagrams of the brachial plexus in the slow and slender lorises, respectively, were given by Harris (1939) and Kanagasuntheram & Jayawardene (1957). There is, as yet, no adequate description of the brain or the limb plexuses of Galagidae. It is therefore hoped that a study of the nervous system in *Galago senegalensis senegalensis* might throw some light on the zoological position of this animal as well as its affinities to *Tarsius*.

The present investigation of the nervous system is confined to a topographical description. It is important to point out here that the collection of the Galagos is often difficult and the preservation of the material in the field is a problem. However, it is hoped that this preliminary investigation will be followed by a detailed histological study when suitable well preserved specimens are available.

MATERIAL AND METHODS

Four brains A, B, C and D have been used in the present study, the first three taken from adult animals and the fourth from a full-term foetus. One spinal cord and three sympathetic trunks were examined, while five cervical, eight brachial and seven lumbo-sacral plexuses were dissected in adult specimens. Furthermore, the peripheral distribution of the terminal branches of these plexuses was observed in two upper and two lower limbs. This entire dissection was done under the binocular dissecting microscope. All animals were fixed in formalin.

OBSERVATIONS

The weights as well as the dimensions of the adult and full-term foetal brains are given in Table 1.

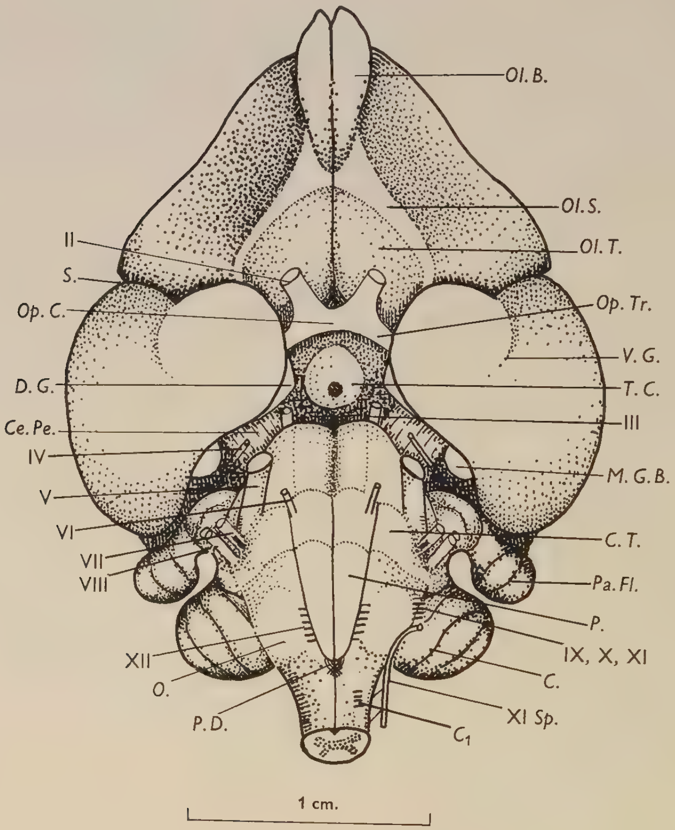
Table 1. *Weights and dimensions of the Galago material examined.*

	Adult brains			D (foetal brains)
	A	B	C	
Weight of animal after fixation (g.)	172	—	—	10·8
Weight of brain after fixation (g.)	5	4·4	—	1·2
Ratio of brain weight to weight of animal	1/36	—	—	1/9
Length of cerebral hemisphere from frontal to occipital pole (mm.)	28·0	26·0	25·0	19·0
Height of cerebral hemisphere (mm.)	15·0	15·0	—	10·0
Maximum width of brain (mm.)	22·0	21·0	22·0	15·0
Length of olfactory bulb (mm.)	9·0	8·5	8·0	5·0
Height of olfactory bulb (mm.)	3·0	4·0	3·0	—
Maximum breadth of olfactory bulb (mm.)	2·0	2·0	2·0	2·0
Protrusion of olfactory bulb beyond frontal pole (mm.)	4·0	4·0	4·0	1·5
Length of corpus callosum (mm.)	9·5	10·0	—	7·5
Antero-posterior length of thalamus along medial surface (mm.)	4·0	5·0	—	3·5
Diameter of medial geniculate body (mm.)	1·5	—	—	—
Diameter of lateral geniculate body (mm.)	2·0	—	—	—
Antero-posterior length of anterior colliculus (mm.)	3·5	4·0	—	2·0
Transverse length of anterior colliculus (mm.)	4·0	—	—	—
Antero-posterior length of posterior colliculus (mm.)	1·5	1·5	—	1·5
Transverse length of posterior colliculus (mm.)	4·0	—	—	—
Antero-posterior length of pons (mm.)	6·0	6·0	—	3·0
Antero-posterior length of medulla (mm.)	6·0	5·5	—	2·5
Transverse length of medulla (mm.)	8·0	8·5	8·0	—
Cranio-caudal length of cerebellum (anterior surface) (mm.)	13·0	11·0	—	6·0
Width of cerebellum at level of para-flocculus (anterior surface) (mm.)	14·0	13·0	—	8·0
Cranio-caudal length of cerebellum (posterior surface) (mm.)	12·0	13·0	—	7·0
Width of cerebellum including para-flocculi (posterior surface) (mm.)	18·0	19·0	—	11·0

The adult brain

The cerebral hemisphere. When the meninges are intact and the brain is still in the skull the cerebral hemispheres almost completely overlap the cerebellum. However, when the meninges are removed there is some displacement so that part of the cerebellum becomes uncovered by the hemispheres.

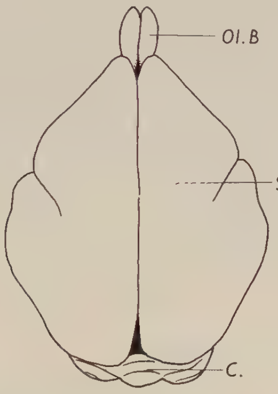
On the ventral surface of the hemisphere (Text-fig. 1) the olfactory bulb is continued posteriorly into the olfactory stria which laterally bounds the olfactory tubercle. There is no rhinal fissure, but the pyriform lobe appears to be separated from the rest of the temporal lobe of the cerebral hemisphere by a vascular groove. The ventral surface of the hemisphere, like the dorsal, is smooth except for the proximal part of the Sylvian fissure and the anterior part of the pre-calcarine fissure. The stem of the Sylvian fissure commences lateral to the olfactory stria as the latter passes backwards to terminate in the pyriform lobe. Behind the temporal pole a part of the dentate gyrus appears along the ventromedial border of the hemisphere, while further posteriorly this border curves outwards to form a concavity containing the medial geniculate body (Text-fig. 1).



Text-fig. 1. Ventral view of brain.

The dorsolateral surface of the cerebrum is smooth except for the Sylvian fissure which runs upwards and backwards and terminates within 8·0 mm. of the superomedial border (Pl. 1, figs. 1, 2 and Text-figs. 2, 4). The occipital pole of the hemisphere is blunter than the frontal pole.

On the medial surface of the hemisphere, the only distinct fissures are the intercalary sulcus and the calcarine system consisting of pre-, retro- and para-calcarine sulci. Their relative positions are shown in Pl. 1, figs. 3, 4 and Text-fig. 3. The relationship of the para-calcarine sulcus to the intercalary sulcus shows some variations. In one adult brain the para-calcarine sulcus is directly continuous with the intercalary sulcus but in another there is a definite discontinuity between the two sulci. The third specimen is intermediate in that the two sulci are joined by a shallow groove. Macroscopic sections of the occipital lobe show the visual stria. The corpus callosum is about a third of the length of the cerebral hemisphere. It has no rostrum but the genu and splenium are represented (Pl. 1, fig. 3 and Text-fig. 5). The anterior commissure is relatively small and lies immediately in front of the anterior column of the fornix. When the hippocampal formation is exposed by the removal of a part of the brain stem, a deep vascular groove is seen to separate the dentate gyrus from the hippocampus and the fimbria. The fimbria, which is a broad flat band under the corpus callosum, narrows considerably as it is traced into the inferior horn of the lateral ventricle; whereas the hippocampus, which commences superiorly as a narrow strip, broadens inferiorly to form the hippocampal tubercle. The dentate gyrus is separated from the rest of the hemisphere by the hippocampal fissure which extends forwards on to the ventral surface of the brain as the fissura amygdaloidea. The latter fissure bounds the anterior part of the dentate gyrus which curves round the hippocampal tubercle, so presenting an appearance similar to the uncus of the human brain. This part of the dentate gyrus is not clearly defined from the hippocampal tubercle and is the only part of the hippocampal formation to appear on the ventral surface of the hemisphere.



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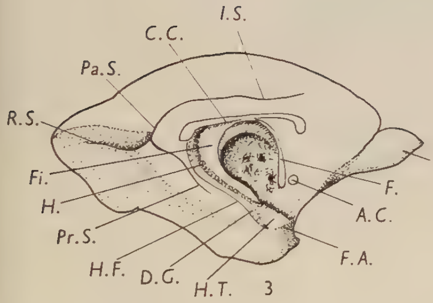

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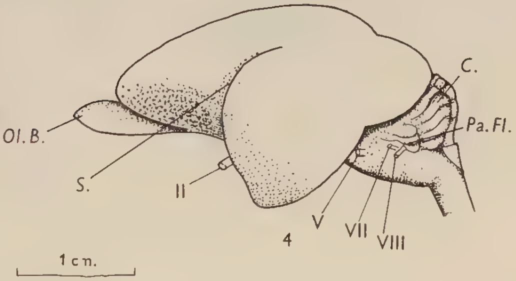

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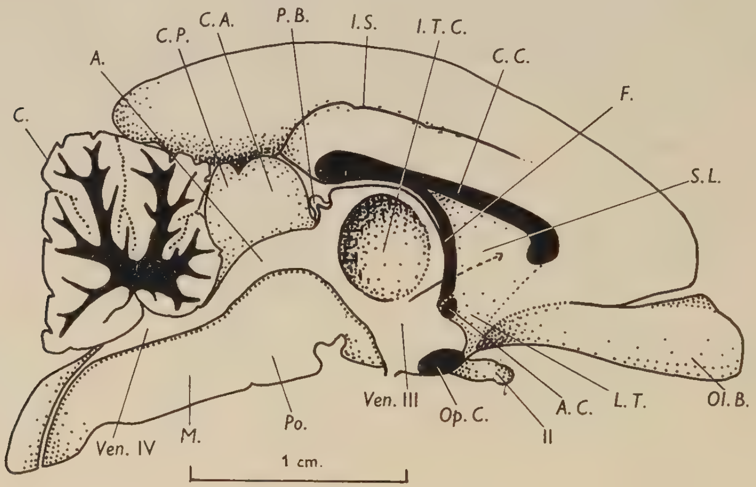
Text-fig. 2. Dorsal view of brain.

Text-fig. 3. Medial aspect of cerebral hemisphere after removal of brain stem.

Text-fig. 4. Lateral view of brain.

The brain stem. The thalamus is large and quadrangular in shape with an extensive interthalamic connexus (Text-fig. 5). The lateral geniculate body is overlapped by the temporal lobe of the hemisphere and not seen in the ventral view of the brain, while the medial geniculate body is visible from this view owing to a deviation outwards of the medial border of the temporal lobe. The anterior and the posterior colliculi are separated by a distinct groove and are in the same horizontal plane as the thalamus. The anterior colliculus is remarkably large, whereas the posterior colliculus is only about half the size of the anterior (Text-fig. 5). The ventral surface of the pons shows a well-marked corpus trapezoideum and a shallow groove runs along the midline in the upper half of the pons, while a relatively deep fissure is present not only in the caudal half of the pons but is also continued into the medulla and spinal cord (Text-fig. 1). The medulla is slightly broader than it is long. In the pons and medulla a distinct pyramid, about 1·5 mm. wide lies close to the midline and presents a decussation at its caudal end. The olive lies lateral to the pyramid in the medulla where it forms a prominent tubercle.



Text-fig. 5. Medial aspect of brain. Arrow through interventricular foramen.

The cranial nerves have the usual exits from the ventral surface of the brain, except the sixth nerve which arises from the anterior part of the groove between the corpus trapezoideum and the pyramid (Text-fig. 1).

The cerebellum. The posterior surface of the cerebellum presents a well-defined vermis and two lateral lobes (Text-fig. 7). In a sagittal section (Text-fig. 8), the fissura prima and secunda are seen to divide the cerebellum into anterior, middle and posterior lobes. The deep fissura prima almost reaches the central core of white matter. In the posterior lobe, the nodule is buried between the dorsal surface of the medulla and the tongue-like expansion of the uvula. The uvula consists of two lobules one of which is subdivided into two folia by a shallow sulcus. The pyramid is separated from the uvula by the fissura secunda and has the same number of lobules and folia as the uvula (Text-fig. 8). The para-flocculus is divided into five folia and is connected to the pyramid by the copula pyramidis which is hidden from the surface by the lateral portions of the middle lobe. The median part of the middle lobe consists of two lobules each divided into two folia. The lateral portions of this lobe are divided into six or seven lobules by transverse sulci which are not in continuity with those of the median part (Text-fig. 7). The anterior lobe is divided by the fissura preculminis into culmen and central lobe, each having two lobules subdivided into five folia (Text-figs. 6, 8). The small lingula rests on the superior medullary velum.

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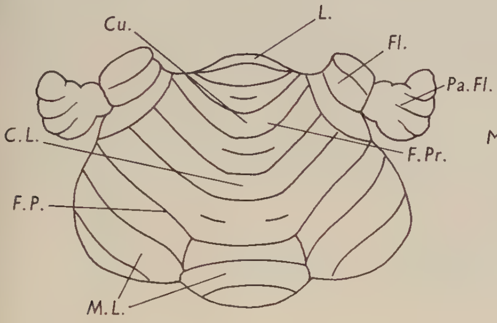

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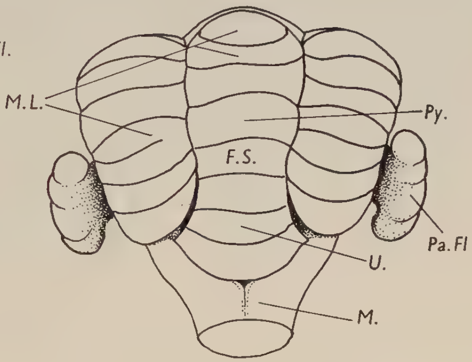

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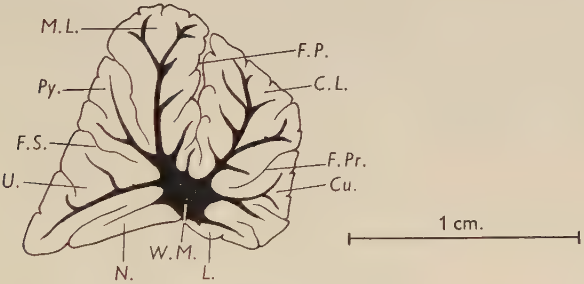


Text-fig. 6. Anterior surface of cerebellum.
Text-fig. 7. Posterior surface of cerebellum.
Text-fig. 8. Sagittal section of cerebellum.

The ventricular system. The topography of this system was difficult to demonstrate by ordinary dissection and it was not until the sagittal section of the brain was stained by the Mulligan method that some details were revealed. The septum lucidum is thick and its cavity could not be found even after a careful search. Although the lamina terminalis could not be defined exactly, it appears to occupy the region between the anterior end of the genu of the corpus callosum above, and the optic chiasma below, while posteriorly it merges with the septum lucidum without any demarcation (Text-fig. 5). The lateral ventricle shows the usual subdivisions but there is no posterior horn. The major part of the third ventricle is confined to the hypothalamic region and a narrow extension of the ventricular cavity surrounds the large interthalamic connexus. The interventricular foramen is represented by a narrow slit, while the supra-optic and pineal recesses are clearly seen. A wide aqueduct connects the third ventricle with the fourth; the latter being roofed by a distinct superior medullary velum.

The full-term foetal brain

The general morphology of the foetal brain is almost identical with that of the adult but relatively more of the cerebellum projects beyond the occipital pole of the cerebral hemisphere. Furthermore, the olfactory portions of the brain are more prominent with relatively larger olfactory bulbs and a thicker anterior commissure. The most striking feature of the cerebral hemisphere is the presence of a large number of deep vascular grooves which are less distinct in the adult. The paracalcarine sulcus is continuous with the intercalary sulcus. It is noteworthy that the pre-, retro- and the para-calcarine sulci diverge from one another leaving between them a well-marked, but shallow, triangular depression (Pl. 1, fig. 3). The dentate gyrus has a well-marked subsplenial flexure which is clearly exposed on the surface whereas, in the adult, this flexure is concealed by the adjacent portions of the hemisphere. The cerebellum shows distinctly the same subdivisions as in the adult.

The spinal cord

The adult spinal cord is about 11·0 cm. in length and ends at the lower border of the sixth lumbar vertebra by tapering rapidly to form the conus medullaris which continues downwards as the filum terminale. The brachial and lumbo-sacral enlargements of the cord are clearly seen and the widest transverse diameters of the cord at these enlargements are 4·0 and 3·5 mm., respectively, whereas it is only 2·5 mm. in the thoracic region.

The peripheral nervous system

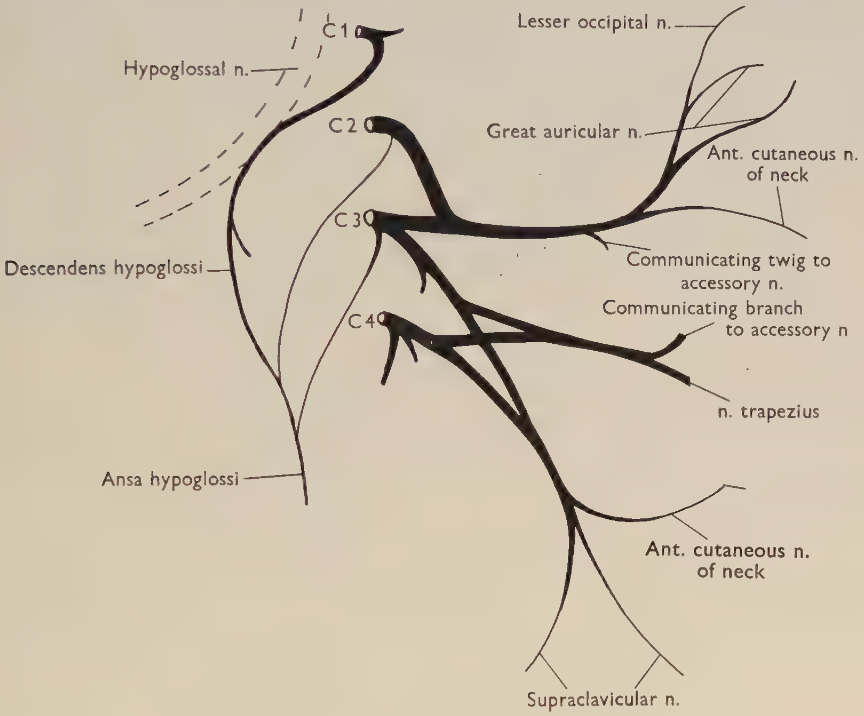
A detailed description of the peripheral nervous system is unnecessary and only illustrations of nerve plexuses and a brief account of some interesting variations are given below.

The cranial nerves. These do not merit much comment. The frontal branch of the ophthalmic division of V does not divide into supra-orbital and supratrochlear branches. The nasociliary nerve is continued into a large infratrochlear nerve which terminates by supplying the external nares. The infra-orbital nerve, emerging through the infra-orbital foramen, is large and communicates with the upper buccal branches of VII. The extra-petrous portion of the facial nerve follows the usual pattern of division found in lower mammals, while the rest of the cranial nerves are similar to those in man.

There are 33 pairs of spinal nerves consisting of 8 cervical, 13 thoracic, 6 lumbar, 3 sacral and 3 coccygeal nerves.

The cervical plexus. In the five cervical plexuses dissected, the distribution is as follows: A common trunk, formed from C2 and 3 passes deep to the sternomastoid muscle and communicates with the accessory nerve (Text-fig. 9). It then divides into branches corresponding to the human lesser occipital and greater auricular nerves. There is no definitive anterior cutaneous nerve of the neck; its place is taken by branches from the great auricular nerve, superiorly, and the supra-clavicular nerve, inferiorly.

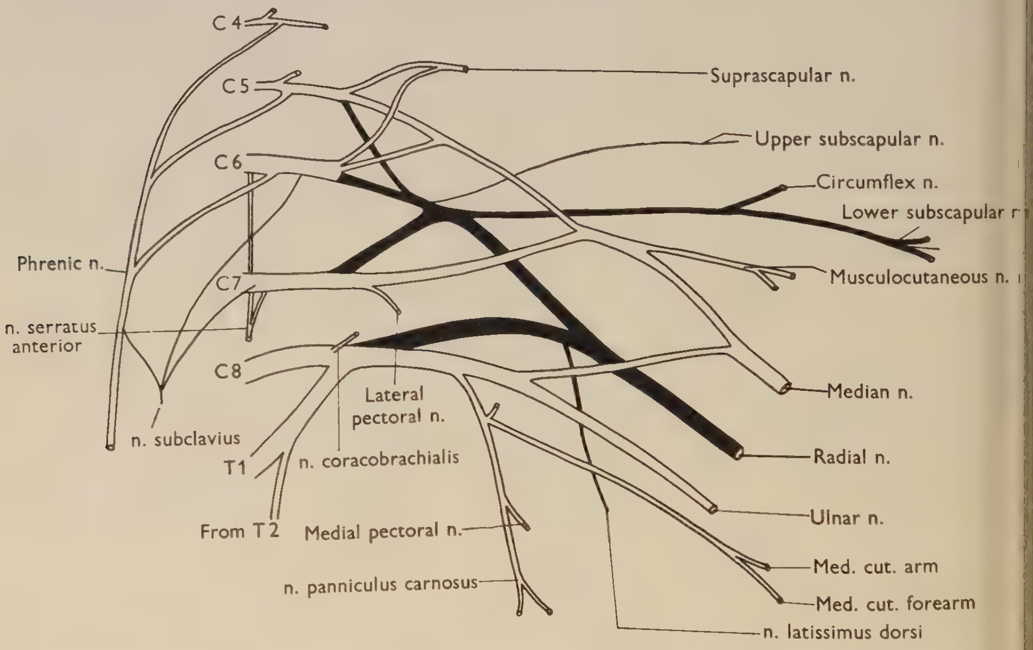
The brachial plexus. All the eight plexuses examined are of the post-fixed type with a large contribution from T2 and a small C5 (Text-fig. 10). C5 and 6 divide into dorsal and ventral branches without forming an upper trunk. There is no definite posterior cord but the medial and lateral cords are formed in the usual manner. The phrenic nerve arises by twigs from roots C4, 5 and 6 and communicates with the vagus nerve and the sympathetic trunk. The nerve to the subclavius muscle arises from the roots C5, 6 and 7 either directly by separate rootlets or by the union of a rootlet from the phrenic nerve with branches arising from C6 and 7 (Text-fig. 10). In two specimens the nerve is formed by the union of a branch from the phrenic with another from C7. It is of interest to note that nerves which, in man, have independent origins from the plexus are here grouped together and arise by a common trunk. For example, the medial pectoral nerve, the medial cutaneous nerves of the arm and forearm and a nerve supplying the panniculus carnosus muscle are all given off from a common trunk arising from the medial cord (Text-fig. 10). Similarly, the circumflex and the lower subscapular nerves arise by a common trunk. The major part of the coracobrachialis muscle is supplied by the musculo-cutaneous nerve, while the



Text-fig. 9. Cervical plexus.

upper coracotuberal portion of the muscle receives a twig from the lower trunk which reaches the muscle after passing deep to the lateral root of the median nerve. The axillary-brachial artery is of the deep type where the median nerve passes superficial to the vessel.

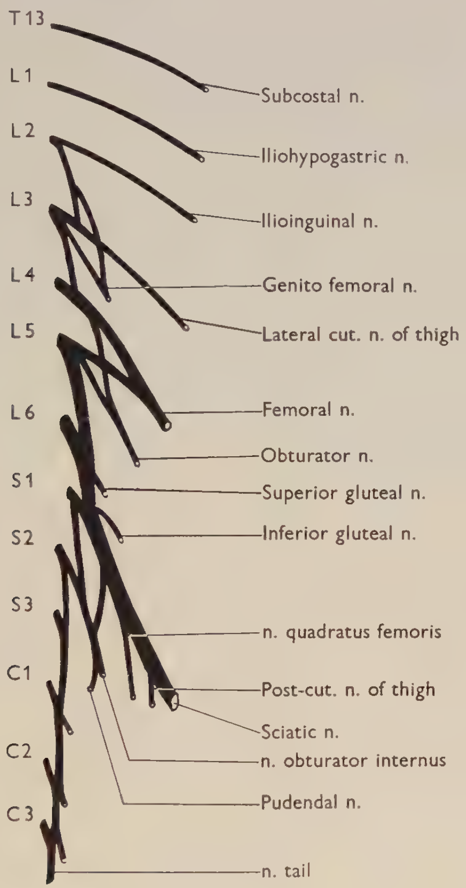
As regards the peripheral distribution in the two upper limbs dissected, the median nerve, in the distal part of the forearm, gives off a branch passing superficial to the flexor retinaculum to supply the thenar musculature as well as the lateral side of the thumb. Furthermore, the cutaneous distribution of the median nerve is reduced to the lateral two and half digits, while there is a corresponding increase in the distribution of the ulnar nerve. However, there is a small communicating twig from the median nerve to the most lateral digital branch of the ulnar nerve. At the elbow, the radial nerve ends by giving off the posterior interosseous nerve; thus *the cutaneous portion of the radial nerve is absent in the forearm.* As a result there is a remarkable alteration in the cutaneous supply of the dorsum of the hand. Branches from the musculo-cutaneous nerve supply the dorsal aspect of the thumb and the radial side of the second finger. The posterior cutaneous nerve of the forearm, a branch of the radial in the spiral groove, provides digital branches to the dorsum of the third, to the radial half of the fourth and ulnar half of the second fingers. The dorsal branch of the ulnar nerve supplies as usual the medial one and half digits.



Text-fig. 10. Brachial plexus.

The lumbo-sacral plexus. All the seven plexuses dissected are of the post-fixed type. The femoral and obturator nerves arise from L4 and 5 (Text-fig. 11). The major part of L5 and 6, together with S1, unite to form a sciatic band from which arise the superior and inferior gluteal nerves, the nerve to the quadratus femoris and gemelli muscles as well as the posterior cutaneous nerve of the thigh. A single trunk divides into the pudendal nerve and the nerve to the obturator internus muscle. The nerve to the tail is remarkably large and is formed by the union of twigs from S 2 and 3 with the three coccygeal nerves.

The terminal branches of the lumbo-sacral plexus follow the same mode of distribution as in man. However, the femoral nerve does not give off separate medial and intermediate cutaneous nerves of the thigh and their area of distribution is taken over by branches from the saphenous nerve and the lateral cutaneous nerve of the thigh. Despite the absence of its femoral head the biceps femoris muscle has a double nerve supply. The posterior tibial nerve, as it passes behind the medial malleolus, gives off a branch which supplies the muscles of the hallux and ends as a cutaneous branch to the medial side of this toe. The anterior tibial nerve ends in the extensor digitorum brevis muscle. Thus the entire cutaneous supply to the dorsum of the toes is taken over by the musculo-cutaneous nerve except for the lateral side of the little toe which receives an additional twig from the sural nerve.



Text-fig. 11. Lumbo-sacral plexus.

The sympathetic trunk. The superior cervical ganglion is bean-shaped with the concavity facing upwards. The lateral pole of the ganglion communicates with the last four cranial nerves and gives off a branch which enters the petrous temporal bone through a small hiatus situated lateral to the jugular foramen. The medial pole of the ganglion, after communicating with the first four cervical nerves and the inferior ganglion of the vagus, passes cranially with the internal carotid artery through the foramen lacerum. No middle cervical ganglion could be found but a large stellate ganglion lies on the neck of the first rib. A striking feature of the thoracic sympathetic trunk is that the splanchnic nerves could not be found even after a careful search. The exact number of ganglia is difficult to define, but five ganglia are clearly visible in the fourth, fifth, sixth, seventh and the twelfth intercostal spaces. Each ganglion receives rami communicantes from more than one intercostal nerve. Branches are given off from the trunk directly to the aorta and the intercostal arteries. The thoracic part of the chain, after passing behind the medial arcuate ligament, has a ganglion which sends a large medial branch to the combined coeliac and superior mesenteric ganglia. Inferiorly, the cord is continued as a slender nerve which, after contributing to the renal and aortic plexuses, receives communications from the first, second and third lumbar nerves and so becomes larger. There are only two visible ganglia in the abdomen. The chain runs downwards medial to the genito-femoral nerve, with which it communicates, and after giving distinct branches directly to all the lumbar arteries finally ends by surrounding the median sacral artery.

DISCUSSION AND CONCLUSIONS

The cerebral hemispheres of *Galago senegalensis senegalensis* are smooth except for the intercalary, the Sylvian and calcarine sulci. Although the brain of *Galago* shows a superficial resemblance to that of *Tarsius* in regard to the paucity of fissures, the configuration of the calcarine system and the Sylvian fissure shows a striking similarity to that of *Microcebus murinus*, a primitive lemur from Madagascar. However, the intercalary sulcus which is so consistent in *Galago*, is absent both in *Microcebus* and *Tarsius*. The intercalary sulcus is continued posteriorly without interruption into the para-calcarine sulcus in the foetal brain as well as in two of the three adult brains. This is of interest in view of the differences of opinion expressed as to the homology of the para-calcarine sulcus, frequently referred to as the parieto-occipital sulcus (Duckworth, 1904). But Elliot Smith (1903) has claimed that the para-calcarine sulcus represents only the ventral element of the more complex parieto-occipital fissure found in higher primates. The occasional continuity of the intercalary with the parieto-occipital sulcus as observed by Kanagasuntheram (1954) in a juvenile Hoolock gibbon gives credence to this view. However, it is suggested that the true homologue of the para-calcarine sulcus is the subparietal sulcus which in the human brain forms the backward continuation of the sulcus cinguli (Wood Jones, 1946). The interposition of the precuneate gyrus between the gyrus cinguli and the visual area of the cortex appears to have displaced the para-calcarine sulcus (subparietal sulcus) forwards with the result that the latter sulcus remains separate from the pre-calcarine and retro-calcarine sulci of the human brain.

Whatever the actual homology of this sulcus may be, it has been concluded by Le Gros Clark (1931) that the pre-calcarine and para-calcarine sulci limit the limbic area in front from the visual area behind. The continuity between the intercalary and the para-calcarine sulci in the brain of *Galago* is remarkable, since neither Elliot Smith (1903) nor Connolly (1950) have noted its presence in true lemurs or even in lorisine lemurs such as *Nycticebus coucang* or *Perodicticus potto*, although the latter animals are included with *Galago* in the same suborder.

The disposition of the calcarine system in the foetal *Galago* brain is of special interest in that a shallow triangular trough of cortex is found where the para-, pre- and retro-calcarine sulci meet. A similar arrangement appears to be present in the brain of *Tarsius* (Le Gros Clark, 1934, fig. 52). But Woollard (1925) does not make special mention of this feature. The absence of this triangular depression in the brain of adult *Galago* suggests that it becomes subsequently overgrown by what is presumably the visual cortex of the occipital lobe. This over-growth recalls the development of opercula over the island of Reil in the human brain.

The Sylvian fissure in *Galago* is composed of the pseudo-Sylvian and supra-Sylvian elements as in *Microcebus* whereas, in *Tarsius*, this fissure is not very conspicuous (Woollard, 1925). Le Gros Clark (1931) states that a true Sylvian fissure is absent in *Tarsius* though a pseudo-Sylvian and a representative of the supra-Sylvian sulcus may be present.

The olfactory portions of the brain in adult *Galago* are not as prominent as in *Microcebus*. The olfactory bulbs show a certain amount of reduction and approach a size intermediate between that of *Tarsius* and *Microcebus* whereas the foetal *Galago* shows closer resemblance to the condition found in *Microcebus*. The same may be said about the dentate gyrus which, in the foetus, shows a well-marked sub-splenial flexure before passing on to the dorsum of the corpus callosum. These differences between the foetal and adult brains suggest a lag in postnatal development of the olfactory system. A further indication of reduction of the olfactory system in *Galago* is the small size of the anterior commissure. Observations of living Galagos (Butler, 1960) indicate that these animals do not rely much on smell but more on sight and hearing, both of which are extremely acute.

The thalamus is quadrangular in shape with the pulvinar forming the postero-medial angle. This contrasts with the rounded organ of insectivores (Le Gros Clark, 1931). The prominence of the lateral geniculate body and the superior corpus quadrigeminum in *Galago* emphasizes the importance of vision in this nocturnal primate. However, the same structures appear to be even more prominent in *Tarsius*.

In *Galago* the cerebellum is almost completely overlapped by the cerebral hemispheres, a feature resembling the lorisoids and *Tarsius* but differing from the true lemurs in which a considerable portion of the cerebellum is exposed beyond the occipital pole. The cerebellum is relatively simple and shows the same subdivisions as in *Tarsius* but it differs in possessing a larger number of folia. This is surprising in view of the saltatory mode of progression adopted by both animals. However, the cerebellum of the gibbon which is the most active brachiator is also found to be the least elaborate among the anthropoids (Kanagasuntheram, 1954). It is, therefore, difficult to correlate the agility of an animal with its macroscopic cerebellar development.

The confluence of the lamina terminalis with a thick septum lucidum, the massive size of the inter-thalamic connexus, the confinement of the third ventricle largely to the hypothalamic region, the considerable width of the aqueduct and the absence of the posterior horn of the lateral ventricle are points of interest with regard to the ventricular system. However, some of these conclusions will have to await further confirmation by histological investigation. The continuity of the lamina terminalis with the septum lucidum in *Galago* is in conformity with known embryological stages in the development of the human brain. A large inter-thalamic connexus as found in *Galago* is also present in both old and new world monkeys such as *Maccacus rhesus* and *Saimiri sciurea* as well as in the gibbon (Kanagasuntheram, 1954).

The proportion of brain weight to weight of the body is 1:34 in *Galago*. This figure is comparable to that of *Microcebus* 1:36, while in *Tarsius* and *Tupais* minor it is 1:26 (Le Gros Clark, 1931). Beattie (1927) quotes the ratio of 1:29 in *Hapale* as the largest among mammals while Wood Jones (1929) states that a ratio far in excess of that reported by Beattie is to be found in some examples of *Lagothrix*. When the weight of the brain is compared with the weight of the fully grown body, it is found that some lower monkeys and small rodents have a ratio which is greater than that in man (Donaldson, 1895). It is, therefore, clear that the significance of these ratios depends upon careful consideration of the weight of the animal.

The brachial plexus in *Galago* is post-fixed which, according to Harris (1939), is usual among lower primates. He observed post-fixed plexuses in *Nycticebus coucang* and *Lemur variegatus* while a similar finding was reported by Kanagasuntheram & Jayawardene (1957) in *Loris tardigradus tardigradus*. It is, therefore, probable that a post-fixed brachial plexus may be the rule in both suborders of the Strepsirhini. But it cannot be assumed that it is solely confined to the lemurs as Woollard (1925) observed a post-fixed plexus in *Tarsius* and Harris (1939) in the marmoset. Le Gros Clark (1926) did not find it in the pen-tailed tree-shrew, although this animal is stated to represent an evolutionary phase in the development of the lemurid form. In *Galago*, C5 and 6 do not join to form an upper trunk, since the dorsal and ventral divisions arise directly from the anterior primary rami. This appears to be common among primates and Harris (1939) found it in *Lemur variegatus*, *Cabus apella*, *Aotus felinus*, *Lagothrix Humboldtii*, *Hylobates leuciscus* and in an example of pre-fixed plexus in man. In *Galago*, the fusion of nerves which have independent origin from the plexus in man, is to be regarded as a primitive feature as is the fusion of the stems of the branches of the axillary artery in lower mammals. The deep type of axillary-brachial artery observed in *Galago* is prevalent in man. The same arrangement was found by Lineback (1933) in *Macacus rhesus* whereas, in the majority of primates, including the great apes, a superficial type of artery is present (Miller, 1939). The communications between the median and ulnar nerves, the musculo-cutaneous and median nerves, and the superficial and deep digital nerves, which are present in *Loris* (Kanagasuntheram & Jayawardene, 1957), are absent in *Galago*. The absence of the radial nerve in the forearm of *Galago* is unique since it has not been reported in any other primate. But Harris (1939) quotes Appleton as having reported an instance in man where the ulnar and musculo-cutaneous nerves supplied the entire dorsum of the hand.

The lumbo-sacral plexus of *Galago*, like its brachial plexus, is post-fixed and consequently most of the nerves arise one segment more caudal than usual. Even so, it is clear from the description of this plexus in *Tarsius* (Woollard, 1925) and in *Ptilocercus* (Le Gros Clark, 1926) that the plexus in *Galago* is distinctly different. For instance, in *Tarsius* there is complete dissociation between the lumbar and sacral portions of the plexus as shown by the absence of a contribution to the sacral plexus from the lowest nerve root taking part in the lumbar plexus. Furthermore, the main contribution to the sciatic nerve in *Galago* is from L 6, whereas in *Tarsius* it is from S 1 and in *Ptilocercus* from L 5. The fusion of nerves, as seen in the brachial plexus of *Galago*, is also present in its sacral plexus: the pudendal nerve and the nerve to the obturator internus arise by a common stem, a feature absent in both *Tarsius* and *Ptilocercus*. Despite the absence of the short head of the biceps femoris in *Galago*, the muscle is innervated by both the medial and lateral popliteal nerves, as observed by Kanagasuntheram (1952) in *Macacus rhesus*. Both in *Galago* and *Tarsius* the musculo-cutaneous nerve supplies almost the entire dorsum of the foot.

In conclusion, it may be stated that in the shape and proportions of the brain, in the absence of a rhinal fissure, in the greater development of the cerebellum and corpus callosum and in the disposition of the sulci, the brain of *Galago* shows a greater resemblance to that of *Microcebus* than that of *Tarsius*. Reduction of the olfactory system, common to *Tarsius* and *Galago*, is also found in various members of the Lorisoidea and consequently has little phylogenetic significance. It may be added that the brain of *Galago* is the most primitive among the Lorisoidea and is surpassed in its simplicity only by the brain of *Microcebus*, the most primitive of the Lemuroidea. The dissimilarity in the arrangement of the sympathetic trunk and the brachial and lumbo-sacral plexuses of *Galago* and *Tarsius* further emphasizes the anatomical differences between them despite their similar mode of progression.

SUMMARY

1. The topographical anatomy of the brain of *Galago senegalensis senegalensis* has been studied in three adults and one full-term foetus. The continuity between the intercalary and the para-calcarine sulci is remarkable. The olfactory system is reduced in size while the visual system, the cerebellum and the corpus callosum are well developed.

2. Five cervical, eight brachial and seven lumbosacral plexuses have been dissected and the distribution of their terminal branches followed in two upper and two lower limbs. The absence of the radial nerve in the forearm is unique.

3. In three sympathetic trunks examined there is a branch from the superior cervical ganglion which enters the petrous part of the temporal bone through a special hiatus. The middle cervical ganglion and the splanchnic nerves are absent and the sympathetic chain ends caudally by surrounding the median sacral artery.

4. The affinities of *Galago* to *Tarsius* and *Microcebus* have been discussed.

We would like to express our thanks to Prof. H. Butler not only for supplying the material used in this study but also for his encouragement and advice. Thanks are also due to Mrs R. Kanagasuntheram for the text-figures, and Mr S J. Woods for the photographs in Plate 1.

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EXPLANATION OF PLATE

Fig. 1. Dorsal view of brain and spinal cord. × 0·9.

Fig. 2. Lateral view of the brain. Note the smoothness of the dorsolateral surface of the cerebral hemisphere. × 1·7.

Fig. 3. Brain as seen in median sagittal section. The intercalary sulcus is shown. Note the absence of the rostrum to the corpus callosum. Compare with Text-fig. 5. × 1·9.

Fig. 4. Cerebrum as seen in median sagittal section. The intercalary, retrocalcarine and paracalcarine sulci are seen. Compare with Text-fig. 3. × 2·9.

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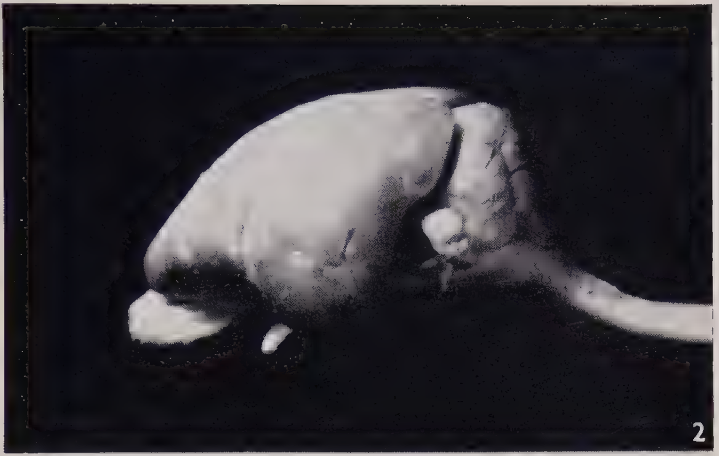

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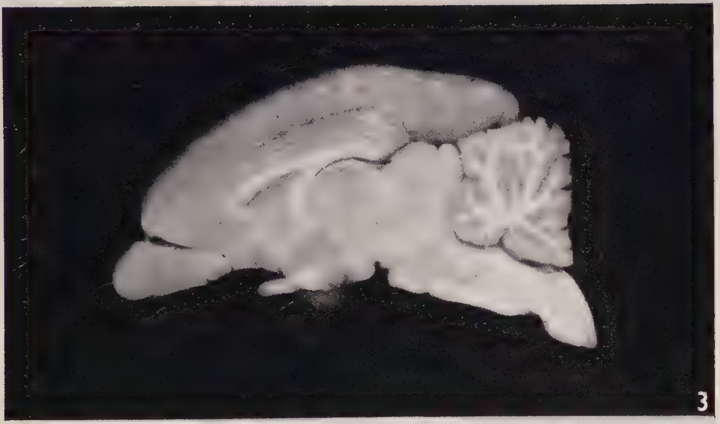

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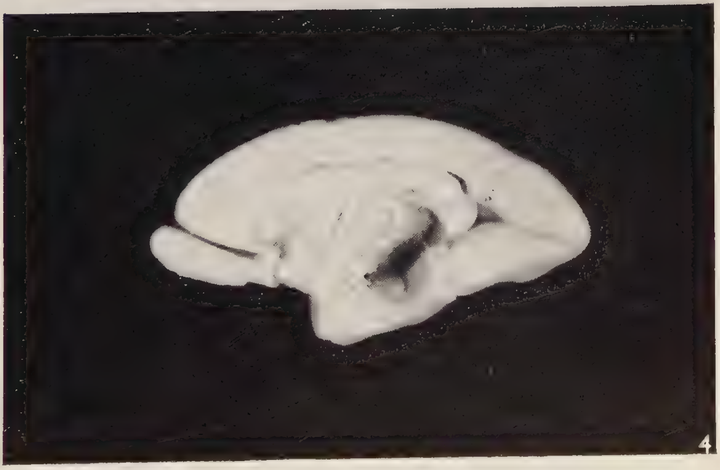

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KANAGASUNTHERAM AND MAHRAN—THE NERVOUS SYSTEM OF THE LESSER BUSH BABY

(*Facing p.* 526)

ABBREVIATIONS

A.	aqueduct
A.C.	anterior commissure
C.	cerebellum
C_1	first cervical anterior nerve roots
C.A.	anterior colliculus
C.C.	corpus callosum
C.L.	central lobe
C.P.	posterior colliculus
C.T.	corpus trapezoideum
Ce.Pe.	cerebral peduncle
Cu.	culmen
D.G.	dentate gyrus
F.	fornix
F.A.	fissura amygdaloidea
Fl.	flocculus
F.P.	fissura prima
F.Pr.	fissura preculminis
F.S.	fissura secunda
Fi	fimbria
H.	hippocampus
H.F.	hippocampal fissure
H.T.	hippocampal tubercle
I.S.	intercalary sulcus
I.T.C.	inter-thalamic connexus
L.	lingula
L.T.	lamina terminalis
M.	medulla
M.G.B.	medial geniculate body
M.L.	middle lobe of cerebellum
N.	nodule
O.	olive
Ol.B.	olfactory bulb
Ol.S.	olfactory stria
Ol.T.	olfactory tubercle
Op.C.	optic chiasma
Op.Tr.	optic tract
P.	pyramid
P.B.	pineal body
P.D.	pyramidal decussation
Po.	pons
Pa.Fl.	para-flocculus
Pa.S.	para-calcarine sulcus
Pr.S.	pre-calcarine sulcus
Py.	pyramid of cerebellum
R.S.	retro-calcarine sulcus
S.	Sylvian fissure
S.L.	septum lucidum
T.C.	tuber cinereum
U.	uvula
Ven. III	third ventricle
Ven. IV	fourth ventricle
V.G.	vascular groove representing rhinal fissure.
W.M.	white matter of cerebellum
II	optic nerve
III	oculomotor nerve
IV	trochlear nerve
V	trigeminal nerve
VI	abducent nerve
VII	facial nerve
VIII	auditory nerve
IX	glossopharyngeal nerve
X	vagus nerve
XI	accessory nerve
XI *Sp.*	spinal root of accessory nerve
XII	hypoglossal nerve

THE THALAMIC PROJECTION UPON THE TELENCEPHALON IN *LACERTA VIRIDIS*

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INTRODUCTION

The basic principles of the organization of the mammalian thalamus have been defined largely as the result of experimental comparative studies in different species of mammals. Thus the afferent and efferent connexions of most of the nuclei of the dorsal thalamus have been established with both anatomical and physiological techniques. It has been shown that all of these nuclei project upon the telencephalon, the majority being related to the neocortex, and the remainder to either the rhinencephalic cortex (Rose & Woolsey, 1943) or to the striatum — directly (Stefens & Droogleever Fortuyn, 1953; Powell & Cowan, 1956*a*) or by way of collaterals (Nashold, Hanbery & Olszewski, 1955).

In contrast to this knowledge of the mammalian thalamus little is known of the connexions of this region in other classes of vertebrates, and what is known has been derived mainly from studies of normal material. The question of the projection of the thalamic nuclei in the bird and reptile is of particular interest because of the radically different structure of the telencephalon in these animals, and especially in view of the relatively greater development of the striatum. In the reptile the striatum can be readily differentiated into a dorsal hypopallial portion, and a ventral palaeostriatum composed of a medial part connected mainly with the hypothalamus and a lateral part connected with the thalamus. Goldby & Gamble (1957) point out, however, that these connexions need experimental confirmation, and that their functional significance is speculative. It has been thought that an extension of experimental investigations similar to those which have been done in the mammal might provide significant data for the interpretation of the phylogenetic development of the dorsal thalamus.

In the present study attention has been directed to the problem of the projection of the thalamus upon the telencephalon in the reptilian brain. The technique of retrograde cell degeneration has been used as it has proved one of the most effective methods for similar studies in the mammal; it may be mentioned that the limitations of the technique are fully realized. The earlier work of Goldby and his associates (Goldby, 1937; Armstrong, 1950; Gamble 1952, 1956; Armstrong, Gamble & Goldby, 1953) on the connexions of the cerebral hemisphere, particularly with the olfactory and visual systems, has encouraged us to employ experimental techniques in the reptile. *Lacerta viridis* has been used in this investigation because it is readily obtainable, and convenient to handle for experimental purposes, and because a detailed description of the cerebral hemisphere has been given by Goldby (1934).

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MATERIAL AND METHODS

Forty-three green lizards (*Lacerta viridis*) were used in these experiments. The operations were performed under ether anaesthesia, and the cerebral hemispheres were exposed by removing parts of the overlying frontal and parietal bones. Lesions of varying size were made in the cerebral hemispheres, either by suction or by a fine needle, with the aid of a binocular dissecting microscope. Many of the lesions were bilateral, so that in all fifty-eight hemispheres were available for study. The opening in the skull was closed with thrombin foam; no signs of infection were found in the brains. The animals were kept at approximately 28° C. for periods ranging from 28 days to 82 days. They were killed by an overdose of ether and the brains fixed in 70 % alcohol and 2 % acetic acid. After embedding in paraffin wax most of the brains were cut in the coronal plane, but some were cut horizontally. The sections were 10μ in thickness; a 1 in 2 series was stained with thionine, and an additional series of many brains was stained by Bodian's protargol method.

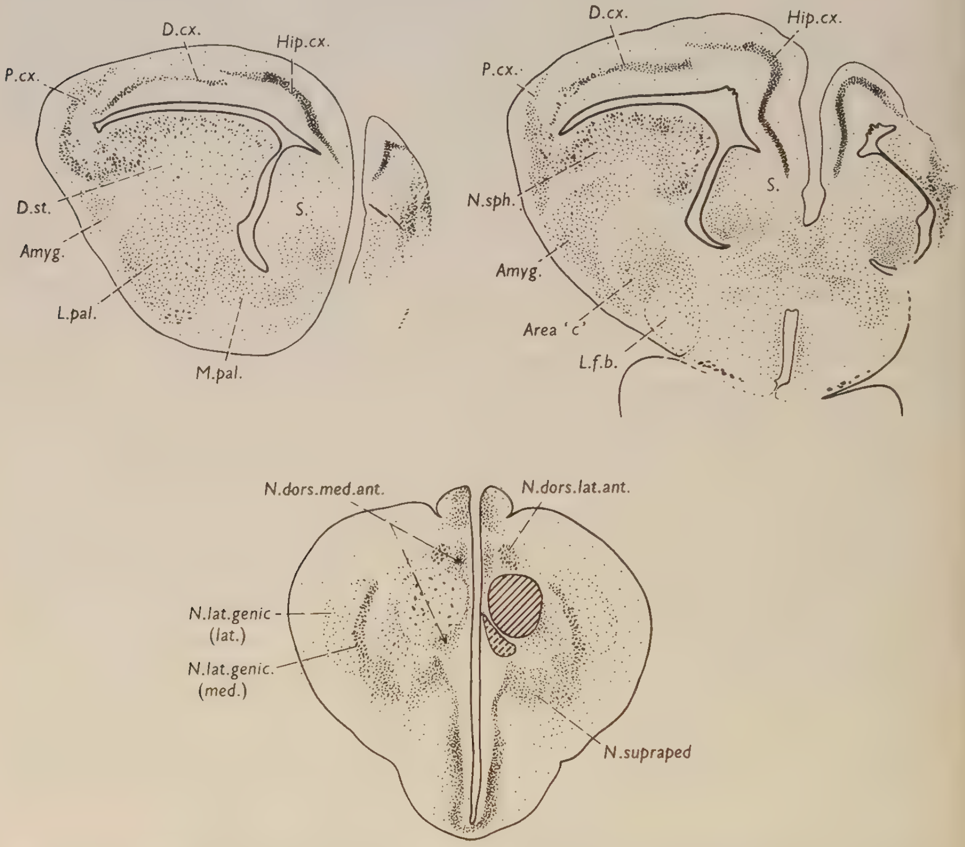
RESULTS

In general the terminology suggested by Goldby & Gamble (1957) in their recent review of the literature of the reptilian cerebral hemisphere has been used in the present study. The thalamic nuclei which will be considered are easily delimited and can be readily identified in the photographs; a well illustrated account of these nuclei in *Lacerta* has been given by Armstrong (1950).

The difficulty in producing localized lesions in the small brain of *Lacerta* has resulted in only a few lesions being restricted to individual structures in the hemisphere. To facilitate the description of the material the lesions have been divided into three groups, and only representative examples of each will be described in detail. In the first group the telencephalon has been almost completely removed; in the second group there has been extensive destruction of the cortex and sub-cortical structures without involvement of the palaeostriatum, and in the last group are the smaller lesions by which the thalamic projection has been more accurately determined.

A striking finding in these experiments in *Lacerta* is that in contrast to mammals most of the thalamic nuclei have survived after removal of the telencephalon. Furthermore, the nature of the retrograde degeneration in the thalamus differs in certain respects from that found in mammals; cell loss is not accompanied by any appreciable glial reaction, and consequently partial degeneration is more difficult to evaluate. The cellular degeneration appears to have reached its maximum by 28 days, and there is no further appreciable change in the appearance of the affected nuclei after 82 days.

In Exp. OL 71 (survival 44 days) which is representative of the first group, the lesion is strictly unilateral, and almost the entire telencephalon has been removed (Text-fig. 1). The anterior third of the right hemisphere has been completely destroyed, and with the exception of a small portion of the medial hippocampal area all the cortex of this hemisphere has been removed, together with the entire dorsal striatum, lateral part of the palaeostriatum, nucleus sphaericus and the central amygdaloid mass. The most rostral part of the remaining tissue consists of the small-celled area of the hippocampal cortex, and more caudally the posterior half of the septum and the ventromedial corner of the hemisphere, including the nucleus of the diagonal band and the medial palaeostriatum, are preserved. The medial palaeostriatum appears to be shrunken, presumably because of the loss of fibres passing through this region, and the cells are swollen. The medial and lateral forebrain bundles have undergone extensive direct involvement. The medial and lateral preoptic areas are intact, but there is marked atrophy of the forebrain bundles at this level.



Text-fig. 1. Drawings made by means of a projection apparatus of transverse Nissl-stained sections to show the extent of the lesion and the distribution of the thalamic degeneration in Expt. OL71. On the operated side only the hippocampal cortex, posterior part of the septum and the ventromedial corner of the hemisphere are preserved.

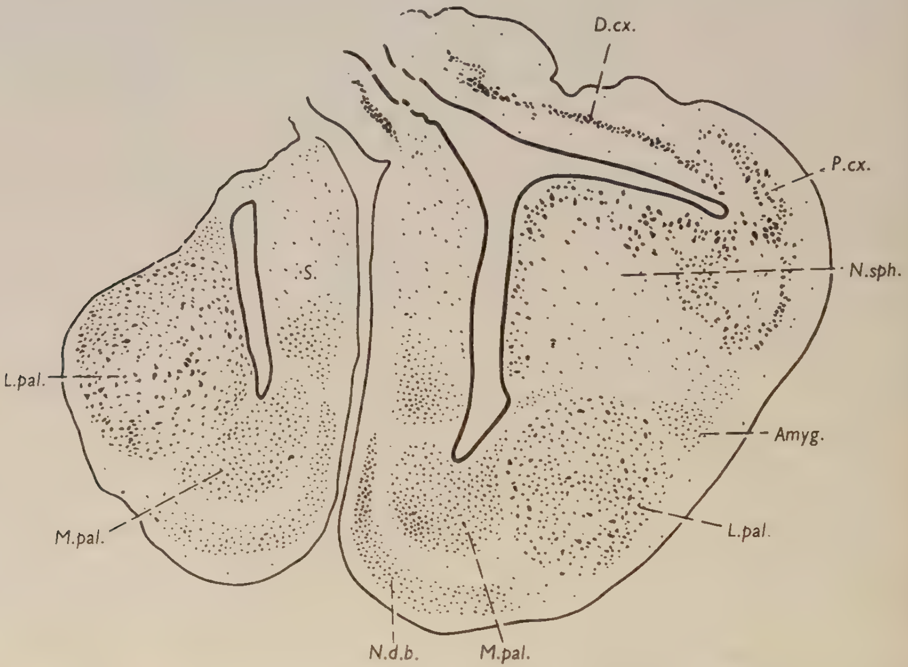
In the thalamus severe retrograde cell degeneration is found in the nucleus rotundus where virtually no normal cells remain. Marked cell loss has occurred, and it is difficult to distinguish the residual shrunken neurons from glial elements. The entire nucleus is involved, but the cell loss is more pronounced in the caudal half. In this brain (as in all others regardless of survival period) retrograde cell degeneration is not accompanied by glial proliferation (Pl. 2. fig. 4; Pl. 3, fig. 5). The only other thalamic nucleus in which degeneration is found is that portion of the nucleus dorsomedialis anterior lying medial and ventral to the nucleus rotundus. In this nucleus there is little, if any, cell loss, but there is distinct shrinkage of all the cells, and the nucleus as a whole is smaller than on the normal side. As no other nuclei show degeneration in this or in any other experiment, the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior appear to constitute the total thalamic projection upon the telencephalon as determined by this technique.

Another experiment (OL 65) is essentially similar to OL 71 except that there is less extensive involvement of the medial palaeostriatum and a little more damage to the septum; in addition there is slight involvement of the dorsolateral margin of the lateral preoptic area. In the thalamus the changes are similar to those described in the previous experiment: the nucleus rotundus shows severe cell loss throughout its extent, and no normal cells remain; in the adjacent part of the nucleus dorsomedialis anterior all the cells are shrunken (Pl. 3, fig. 5).

In an additional series of sections of this brain stained with Bodian's protargol method the thalamic components of the lateral forebrain bundle have been studied. On the normal side three separate groups of fibres can be seen passing from the dorsal aspect of the lateral forebrain bundle to enter the thalamus. In the rostral part of the diencephalon an anterior component is given off which first passes backwards and upwards and then forwards to reach the anterior parts of the dorsal nuclei. More posteriorly an intermediate component passes upwards and medially to the nucleus rotundus and the nucleus dorsomedialis anterior, and at a slightly more posterior level a medial component passes medial to these nuclei. This description agrees closely with that given for these fibre bundles in the alligator brain by Huber & Crosby (1926), but it has not been possible to distinguish in *Lacerta* the internal component described by these authors in the alligator. It should be noted that immediately posterior to the level at which the intermediate component is given off to the nucleus rotundus the dorsal peduncle of the lateral forebrain bundle undergoes a marked decrease in size, but the ventral peduncle can be clearly traced backwards through the diencephalon to the mid-brain.

On the operated side of this brain both the medial and lateral forebrain bundles show severe fibre loss, but this is more marked in the lateral bundle, the lateral two-thirds of which are almost totally devoid of fibres at the level of the preoptic areas (Pl. 3. fig. 6). Some fibres remain in the medial one-third of the lateral forebrain bundle, and these fibres can be seen to join (or leave) the bundle as it passes through the lateral preoptic area. More posteriorly in the diencephalon clear spaces sharply outline the degenerated dorsal and ventral peduncles. The anterior and intermediate thalamic components of the dorsal peduncle are severely degenerated, and only a small proportion of the normal fibres remain (Pl. 4, figs. 7, 8). The medial component, however, appears to be unchanged. The dense fibre plexus of the nucleus rotundus is not appreciably reduced, and in the posterior part of the nucleus the afferent fibres from the tecto-thalamic tract can be seen passing in to form the main contribution to this plexus.

The results of this experiment indicate that the axons of the cells of the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior pass into the intermediate component of the lateral forebrain bundle to reach the telencephalon, but they do not preclude the possibility that there are also descending fibres in this component. The profound degree of degeneration of the anterior thalamic subdivision, taken together with the absence of any retrograde cell degeneration in the anterior parts of the nuclei dorsomedialis anterior and dorsolateralis anterior, strongly suggests that this subdivision is made up largely of fibres which arise in the telencephalon and pass to terminate in these nuclei.



Text-fig. 2. Drawing traced from a section to show the extent of the lesion in Expt. OL 73. The palaeostriatum is completely preserved as the ventral limit of the lesion coincides with the dorsolateral margin of the lateral palaeostriatum.

The second group of experiments indicates that those nuclei which project upon the telencephalon do not project upon the cortex, dorsal striatum and nucleus sphaericus. The best example is experiment OL 73 (survival 80 days) in which no thalamic degeneration is found after extensive involvement of these telencephalic structures (Text-fig. 2). The lesion in the left hemisphere begins near the anterior pole of the hemisphere where it involves the dorsolateral surface, and it rapidly increases in extent so that all three cortical areas, the entire dorsal striatum and the nucleus sphaericus are destroyed. The extent of the lesion remains essentially the same up to the caudal end of the hemisphere where a small portion of hippocampal cortex is preserved. Throughout its antero-posterior extent the ventral limit of the lesion coincides with remarkable precision to the dorsolateral border of the lateral palaeostriatum (Pl. 1, fig. 1). Medially the lesion reaches the junction of the hippocampal cortex and the septum. The septum is not directly involved, but is slightly shrunken and shows compacting and apparent increase of cells, presumably the result of atrophy of fibres of passage. The preoptic areas are also intact. In addition, there has been slight damage to the dorsomedial margin of the right hemisphere.

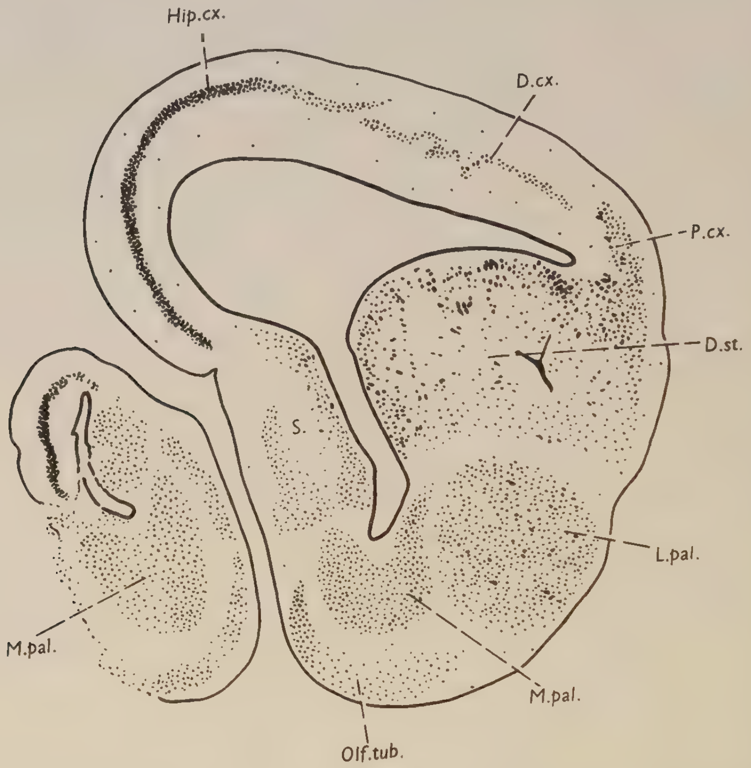
In several other experiments similar but smaller lesions in the cerebral hemisphere have not resulted in any thalamic degeneration.

The Bodian-stained sections of this experiment show that a distinct loss of fibres has occurred in the lateral half of the lateral forebrain bundle at the level of the preoptic areas. The medial and intermediate thalamic components show no appreciable change, but there has been a distinct loss of fibres from the anterior component. The severity of this degeneration is not as marked as in the previous experiment, but it would appear that about half of the normal complement of fibres has degenerated. From a correlation of the extent of the lesion with the fibre loss in this and the previous experiment it is apparent that the intermediate thalamic component of the lateral forebrain bundle is intimately related to either the palaeostriatum or the amygdala because the preservation of this component in Exp. 73 is undoubtedly due to the lack of involvement of these more ventrally situated telencephalic structures. Moreover, it may also be concluded that if this component of the lateral forebrain bundle contains fibres descending from the telencephalon to the thalamus these fibres must arise in these two structures. The finding that no appreciable number of fibres of the intermediate component has degenerated after such an extensive lesion suggests that the thalamic projection upon the telencephalon is localized rather than diffuse. As the severity of the degeneration of the anterior component in this experiment is not as marked as in OL 65 it is clear that this bundle receives fibres partly from the palaeostriatum and amygdala on the one hand and partly (at least one-half) from the dorsal striatum, nucleus sphaericus and cortex on the other hand.

In seven other experiments with smaller lesions involving these three more dorsally placed structures both thionine and Bodian series have been prepared. In all of these brains the medial and intermediate components are found to be unaffected, but the anterior component shows varying degrees of fibre loss. The severity of this loss is proportional to the extent of the damage to the dorsal striatum and nucleus sphaericus, and minimal degeneration is seen in those brains in which the striatal involvement is slight. In three further experiments the lesion is virtually restricted to the cortex, and in these brains Bodian-stained sections show no appreciable change in the lateral forebrain bundle or in any of its thalamic components. These results indicate that in *Lacerta* the number of fibres which enter or leave the dorsal cortex from the lateral forebrain bundle is very small, and this agrees with Goldby's findings (1937).

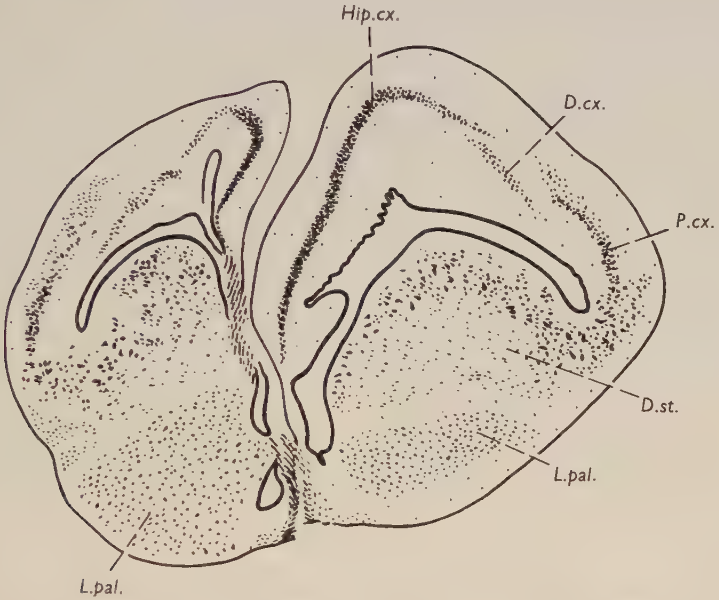
If the thalamic projection is localized rather than diffuse, it would follow from the results of the preceding experiments that the termination of the thalamic axons must be the palaeostriatum or the central amygdaloid mass. The first experiment of the third group (OL 66, survival 28 days) not only excludes the medial part of the palaeostriatum but also shows that degeneration in the thalamus occurs after involvement of the lateral palaeostriatum and the central amygdaloid mass. The central amygdaloid mass is that group of cells found dorsal and posterior to the lateral palaeostriatum and described in detail by Goldby (1934); it is morphologically distinct from the nucleus sphaericus with which it is often grouped as the reptilian homologue of the mammalian amygdala.

The lesion in OL 66 has destroyed the frontal pole of the hemisphere and has severed the olfactory peduncle (Text-fig. 3). The ventromedial corner of the hemisphere reappears at the level of the rostral end of the medial palaeostriatum and slightly more caudally the septum and hippocampal cortex are preserved. Back to the level of the preoptic areas, the structures which are intact include virtually the entire medial palaeostriatum, the nucleus of the diagonal band, septum and the small-celled portion of the hippocampal cortex. The septum shows the same features of cellular compacting as were described in OL 73. The lesion extends posteriorly to completely destroy the posterior pole of the hemisphere, but the preoptic areas and the forebrain bundles are not directly involved. The thalamic degeneration is restricted to severe cell loss in the nucleus rotundus and marked shrinkage of the cells of the adjacent portion of the nucleus dorsomedialis anterior (Pl. 2. fig. 4). The only structures involved by the lesion in this experiment which are not destroyed in OL 73 (in which no degeneration is found) are the lateral palaeostriatum and central amygdaloid mass.



Text-fig. 3. Drawing traced from a section of the cerebral hemispheres of Expt. OL 66. The medial part of the palaeostriatum has not been damaged on the operated side, and the other structures which are preserved include the hippocampal cortex, the septum and the nucleus of the diagonal band.

The next experiment of the third group to be described strongly suggests that the thalamic degeneration results from destruction of the lateral palaeostriatum. In animal OL 67 (survival 28 days) there is a bilateral lesion involving different portions of the palaeostriatum on each side but with unilateral retrograde degeneration in the thalamus. This lesion has been produced by a needle inserted between the two hemispheres resulting in asymmetrical destruction of the ventromedial regions of both sides (Text-fig. 4). In the right hemisphere the damage begins close to the frontal pole medial to the ventricle, and the medial hippocampal cortex and olfactory tubercle are destroyed. The medial margin of the dorsal striatum is slightly involved only near its rostral extremity, and further caudally the entire extent of the ventricular margin of the striatum is intact. The damage is restricted to the medial and lateral palaeostriatum ventral to the inferior angle of the ventricle, the dorsolateral part of the lateral palaeostriatum being preserved. The damage remains restricted to the palaeostriatum, and posteriorly the lesion ends abruptly at the level of the appearance of the nucleus of the diagonal band. It should be emphasized that the central amygdaloid mass, together with the cells capping the lateral forebrain bundle, are uninvolved. The lateral forebrain bundle shows slight atrophy. The retrograde cell degeneration in nucleus rotundus is less severe than in the experiments previously described but is quite definite. There is little cell loss, but all the cells are considerably shrunken. Similar changes have occurred in the adjacent portion of the nucleus dorsomedialis anterior. On the left side only the medial palaeostriatum has been destroyed, below and medial to the lateral ventricle over the same antero-posterior extent as the damage on the right side. There is no involvement of the lateral palaeostriatum. No retrograde cell degeneration is seen in the thalamus on this side.



Text-fig. 4. Drawing traced from a section to show the asymmetrical damage to the cerebral hemispheres in Expt. OL 67. On the left side the lateral palaeostriatum is uninvolved, but on the right side only the dorsal part of this structure is preserved.

The absence of degeneration on the left side after such a lesion again excludes the medial palaeostriatum as a projection area of the degenerated thalamic nuclei. Furthermore, the lack of involvement of the amygdaloid complex by the lesion on the right side of this experiment indicates that this structure can be included with those regions which have already been eliminated as the thalamic projection area in previous experiments. It is possible that fibres do pass from the thalamus to the amygdaloid complex through the adjacent lateral palaeostriatum, and that the degeneration in this experiment could be interpreted as being the result of the interruption of these fibres. This possibility appears unlikely, however, unless the fibres first pass anteriorly through the lateral palaeostriatum and then curve back to the amygdaloid complex. The findings in this experiment, taken together with those previously described, show that the only common factor in all lesions resulting in thalamic degeneration is involvement of the lateral palaeostriatum.

The final experiments of this group indicate that small lesions of the lateral palaeostriatum result in more restricted thalamic degeneration. In animal OL 52 (survival 28 days) only the posterior part of the lateral palaeostriatum has been involved, and partial degeneration is found in nucleus rotundus. In the anterior half of the hemisphere the hippocampal and general cortex have been destroyed, and the lateral ventricle opened. Although it has not been directly involved, the dorsal striatum is severely atrophied. Immediately rostral to the nucleus of the diagonal band the needle used to make the lesion has passed through the inferior angle of the ventricle into the dorsal part of the medial palaeostriatum. The damage to the medial palaeostriatum does not increase posteriorly, but the caudal half of the lateral palaeostriatum and the overlying dorsal striatum at this level have been completely destroyed. The central amygdaloid mass is extensively involved, but the cells overlying the lateral forebrain bundle (Goldby's area *C*) and the nucleus sphaericus are preserved. The damage to the cortex progressively diminishes in the caudal half of the hemisphere. The anterior half of the nucleus rotundus shows no appreciable change, but in the posterior half there is severe cell shrinkage and partial cell loss. The nucleus dorsomedialis anterior adjacent to the degenerated portion of nucleus rotundus shows no distinct changes, but in the anterior half (adjacent to the undegenerated part of nucleus rotundus) the cells appear shrunken. In another experiment, OL 89 (survival 82 days) a small lesion has destroyed the posterior part of the lateral palaeostriatum together with the immediately adjacent part of the central amygdaloid mass and the dorsal striatum. This brain has been cut in the horizontal plane so that the cell shrinkage, which has occurred in the nucleus rotundus and the adjoining nucleus dorsomedialis anterior as a result of this circumscribed area of damage, is clearly seen (Pl. 2, fig. 3).

In three other brains (OL 80, 87 and 93), in which there was less extensive involvement of the posterior part of the palaeostriatum, partial retrograde cell degeneration has occurred in the nucleus rotundus, the cells of the posterior half of the nucleus being considerably shrunken. In two of these experiments an additional series of Bodian-stained sections has been available. These series show that the anterior thalamic component of the lateral forebrain bundle has undergone severe degeneration, and that although the rostral part of the intermediate component is unaltered its caudal part is partially degenerated.

DISCUSSION

From the results of the present experiments it would appear that in *Lacerta viridis* the total thalamic projection upon the telencephalon, as determined by the method of retrograde cell degeneration, consists of the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior. The cells of these two nuclei could either project diffusely to make contact with several structures through terminal branching of their axons or by way of collaterals. From a correlation of the site and extent of the lesion on the one hand with the thalamic degeneration on the other it may be concluded that the projection is restricted to the lateral part of the palaeostriatum. Thus damage of the lateral part of the palaeostriatum is always found to result in cellular degeneration in these two nuclei, but no changes are found after extensive lesions involving most of the other structures of the cerebral hemisphere. The palaeostriatum is generally subdivided into a medial 'olfacto' part connected mainly with the hypothalamus and a lateral 'somatic' part connected with the thalamus. The present experimental results show that this morphological subdivision is justified in so far as the two parts have differential connexions from the thalamus.

In those experiments in which the involvement of the lateral palaeostriatum is circumscribed partial degeneration is found in the thalamic nuclei. The distribution of the degeneration varies with the position of the lesion, and the areas affected in the two nuclei vary independently of each other. Although this evidence is derived from few experiments, and partial degeneration is difficult to evaluate because of the absence of accompanying gliosis, it does suggest that there is a topical organization in the thalamic projection in the antero-posterior direction at least, and also that the two nuclei project independently.

There is a distinct qualitative difference in the reaction of the cells in the nucleus rotundus and in the nucleus dorsomedialis anterior after removal of the telencephalon. The degeneration in the nucleus rotundus is the more severe and appreciable cell loss occurs, whereas only cell shrinkage is apparent in nucleus dorsomedialis anterior. Such differences in cellular reaction almost certainly reflect distinct differences of axonal distribution, and the persistence of the cells in nucleus dorsomedialis anterior suggests that they give off collateral branches to other parts of the diencephalon or brain stem.

Although the nucleus dorsomedialis anterior in *Lacerta viridis* cannot be subdivided on the basis of normal morphology it can be clearly differentiated into an anterior and posterior portion on the basis of differences of the reaction of the cells in these portions to removal of the telencephalon. The main anterior part of the nucleus shows no appreciable changes, but in the posterior extension of the nucleus lying medial to the nucleus rotundus the cells show definite shrinkage. The results of these experiments indicate, therefore, that these two parts of the nucleus have different efferent connexions and are also probably distinct functionally. In the corresponding position in the thalamus of the alligator Huber & Crosby (1926) have differentiated two nuclei on the basis of their morphological differences, the nucleus dorsomedialis anterior, and more posteriorly, adjacent to the nucleus rotundus, the nucleus medialis which in turn could be subdivided into an anterior and a posterior part. These authors conclude that few, if any, of the fibres of the lateral forebrain bundle are related to the nucleus dorsomedialis anterior, but that the nucleus medialis anterior is connected with the striatum through the intermediate thalamic component of this bundle and the nucleus medialis posterior through the medial component. It would appear that the nucleus dorsomedialis anterior of *Lacerta viridis* is homologous with the more differentiated nucleus medialis anterior of the alligator on the basis of its position and connexions.

The simplest interpretation of the absence of cellular degeneration in other thalamic nuclei is that these nuclei do not project upon the telencephalon. In general it may be stated that most of the comparable evidence from experimental work on the thalamic projection in the mammal would support such an interpretation. An important corollary of this interpretation would be the absence of a thalamic projection to telencephalic structures other than the palaeostriatum, and in particular the general cortex. Because of the limitations of the method of retrograde cell degeneration, and especially in view of the limited experience of this technique in the reptile, this lack of cellular change cannot be considered to exclude completely the possibility of an additional projection to the telencephalon from these nuclei. The axons of the cells of these nuclei could terminate in the telencephalon, but a collateral may be given off to the diencephalon or to the brain stem which may be sufficient to maintain the integrity of the cell. It would be of interest to determine which of these possibilities is correct by tracing the course and termination of the degenerating fibres following lesions in the thalamus.

The findings in the Bodian-stained sections must also be interpreted with caution as this technique only demonstrates fibre loss of considerable degree. The present observations are concerned only with the lateral part of the basal forebrain bundle and its dorsal peduncle and thalamic components; no reference will be made to any changes in other tracts or commissures. The results of these experiments indicate that the fibres in the three thalamic components have different origins. The medial thalamic component shows no appreciable change after virtually complete removal of the telencephalon, which suggests that its constituent fibres arise and terminate in the diencephalon or brain stem. The intermediate thalamic component only degenerates if the palaeostriatum is involved, and the close correlation between the fibre degeneration in this component and the cellular degeneration in the nucleus rotundus and nucleus dorsomedialis anterior—whether total or partial—makes it highly probable that this component is composed largely of the efferent fibres from these nuclei. The anterior thalamic component is related to both the palaeostriatum and the dorsal striatum, as it shows almost complete atrophy after destruction of both these structures and a loss of approximately one half of its fibres after involvement of the dorsal striatum only; few, if any, of the fibres of this component are related to the cortex. The absence of any cellular change in the closely related anterior group of thalamic nuclei strongly suggests that the fibres of this subdivision are descending from the striatum to the thalamus. In this discussion of the constitution of the lateral forebrain bundle the palaeostriatum has not been subdivided into medial and lateral parts as there is insufficient evidence to justify this distinction. If the results on the fibre loss are considered in the light of the retrograde cell degeneration findings, however, it is clear that the intermediate thalamic component, at least, is related to the lateral palaeostriatum.

In general, these conclusions agree with those derived from the study of normal material (Kappers, Huber & Crosby, 1936) because it has long been known that the lateral forebrain bundle forms the main pathway between the striatum and the thalamus, but it has not been possible to be certain of the direction of conduction of the fibres. They are also in general accord with the experimental work of Goldby (1937) using the Marchi technique. Goldby concludes that few fibres from the cortex enter the lateral forebrain bundle, that most of the fibres in this bundle are efferent from the thalamus and that the majority of them end in the striatum. The two differences between his conclusions and those presented above are not serious. Firstly, the apparent absence of degeneration in the anterior thalamic component in the Marchi material is probably to be explained in part by the fact that Goldby is referring mainly to that part of the dorsal peduncle related to the nucleus rotundus (where fibre degeneration would not be expected), and partly because, as he emphasizes, the Marchi technique only demonstrates degenerated myelinated fibres. Secondly, although Goldby concludes that most of the efferent fibres from the thalamus end in the striatum he considers that they are particularly related to the hypopallial part; he states, however, that the degree of this degeneration is relatively slight, and secondary to a direct lesion of the lateral forebrain bundle and palaeostriatum so that the origin of these ascending fibres is not certain, and they could have arisen from the palaeostriatum itself.

The most significant result of the present series of experiments is the finding that the thalamic projection upon the telencephalon in *Lacerta* is restricted to the lateral part of the palaeostriatum. This result is particularly surprising in view of the descriptions from normal material of thalamic efferent connexions with other parts of the striatum (Kappers, Huber & Crosby, 1936), and it immediately raises the problem of the origin of the afferent connexions to other structures such as the dorsal striatum, the nucleus sphaericus and the dorsal cortex. The finding of a restricted thalamic projection in the lizard is strikingly similar to that obtained with the same technique in the avian brain where it has been shown that most of the thalamic nuclei (including the nucleus rotundus and the nucleus ovoidalis), which degenerate after removal of the telencephalon, are related to the palaeostriatum augmentatum (Powell & Cowan, 1957). On the basis of their morphology and of their connexions one might therefore be tempted to suggest that the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior of the lizard are homologous with the nucleus rotundus and nucleus ovoidalis of the bird, and that the lateral part of the palaeostriatum of the reptilian brain is the homologue of the avian palaeostriatum augmentatum. The question as to which structures are homologous in the brains of *modern* reptiles and birds is much more complex than this, however, and it is probably sufficient to say that in using as a criterion the fibre connexions between two structures it is not necessarily to be inferred that the thalamus or the palaeostriatum of the reptile is homologous in its nuclear components with the corresponding regions in the bird. Experimental evidence of the afferent connexions of the thalamic nuclei in both groups of animals and of the other afferent and efferent connexions of the palaeostriatum would help to clarify this problem. The conclusion that may be drawn is that in both the reptilian and avian brain the telencephalic projection of the thalamus is predominantly to the palaeostriatum. This similarity of the thalamic projection is perhaps not unexpected in view of the closely related evolutionary history of the two groups and the generally accepted view of the close resemblance between the brains, the avian brain differing mainly in the degree of development of the striatum. As Goldby & Gamble (1957) have recently pointed out, even more difficulty would be encountered if the attempt were made to draw homologies between the reptilian and mammalian brain where the thalamus is known to be intimately related to the neocortex. On the one hand, there is no evidence to indicate whether the palaeostriatum of the reptile is homologous with the globus pallidus or the caudate nucleus and putamen of the mammal, and on the other hand the reptilian thalamus may be the homologue not of the mammalian dorsal thalamus but of one of the other main subdivisions of the diencephalon such as the subthalamus. That the thalamus of these two classes is probably organized in a fundamentally different manner is suggested by the differences which have been found in the central connexions of the optic nerve. The dorsal nucleus of the lateral geniculate body of the mammal receives optic nerve fibres, and its cells either project upon the neocortex or are interneurons; there is no unequivocal evidence to indicate that the ventral nucleus of the lateral geniculate body receives optic nerve fibres. The lateral geniculate nucleus of *Lacerta* has also been shown to be the site of termination of fibres of retinal origin (Armstrong, 1950) but observations on normal material have shown that its predominant efferent connexions are with the tectum of the midbrain and not with the telencephalon, and these observations are in agreement with the findings of the present investigations in which no retrograde cell degeneration has been found in the nucleus after removal of the telencephalon. One may speculate as to whether the predominant striatal projection of the thalamus which has been found in the reptile and bird may not form the basic pattern, upon which the close thalamo-cortical relationship of the mammalian dorsal thalamus has been superimposed as a specialized evolutionary development.

SUMMARY

1. The projection of the thalamus upon the telencephalon in *Lacerta viridis* has been investigated using the method of retrograde cell degeneration. A study of Bodian-stained sections of the same experimental material has provided evidence of the origin of certain components of the lateral forebrain bundle.

2. After virtually complete removal of the telencephalon retrograde cell degeneration in the thalamus is restricted to the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior.

3. Analysis of the retrograde cell degeneration in the thalamus following lesions of varying size has shown that these nuclei project to the lateral palaeostriatum.

4. The intermediate thalamic component of the lateral forebrain bundle consists largely of the efferent fibres from the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior to the lateral palaeostriatum. The anterior component is composed of fibres which arise from the dorsal striatum, nucleus sphaericus and palaeostriatum. There are few, if any, fibres in the lateral forebrain bundle which are related to the dorsal cortex.

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ABBREVIATIONS

A.	Anterior thalamic component of lateral forebrain bundle.
Amyg.	Central amygdaloid mass.
Area 'c'	Area *C* of the amygdala described by Goldby (1934)
D.cx.	Dorsal cortex.
D.st.	Dorsal striatum.
Hip.cx.	Hippocampal cortex.
I.	Intermediate thalamic component of lateral forebrain bundle.
L.	Lateral forebrain bundle.
L.f.b.	Lateral forebrain bundle
L.pal.	Lateral palaeostriatum.
M.	Medial forebrain bundle.
M.pal.	Medial palaeostriatum.
N.d.b.	Nucleus of the diagonal band.
N.dors.lat.ant.	Nucleus dorsolateralis anterior.
N.dors.med.ant.	Nucleus dorsomedialis anterior.
N.lat. genic. (lat.)	Lateral part of lateral geniculate nucleus.
N.lat. genic. (med.)	Medial part of lateral geniculate nucleus.
N.sph.	Nucleus sphaericus.
N.supraped.	Suprapeduncular nucleus.
Olf.tub.	Olfactory tubercle.
P.cx.	Pyriform cortex.
R.	Nucleus rotundus.
S.	Septum.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Photomicrograph showing the extent of the lesion in Expt. OL 73; note the complete preservation of the lateral palaeostriatum. Stained with thionine. × 36.

Fig. 2. Photomicrograph to show retrograde cell degeneration in the nucleus rotundus and the adjacent part of the nucleus dorsomedialis anterior in Expt. OL 56. Stained with thionine. × 40.

Plate 2

Fig. 3. Photomicrograph of a horizontal section to show severe cell shrinkage in the nuclei rotundus and dorsomedialis anterior in Expt. OL 89. Stained with thionine. × 69.

Fig. 4. Photomicrograph showing severe cell loss in nucleus rotundus and cell shrinkage in nucleus dorsomedialis anterior in Expt. OL 66. Stained with thionine. × 87.

Plate 3

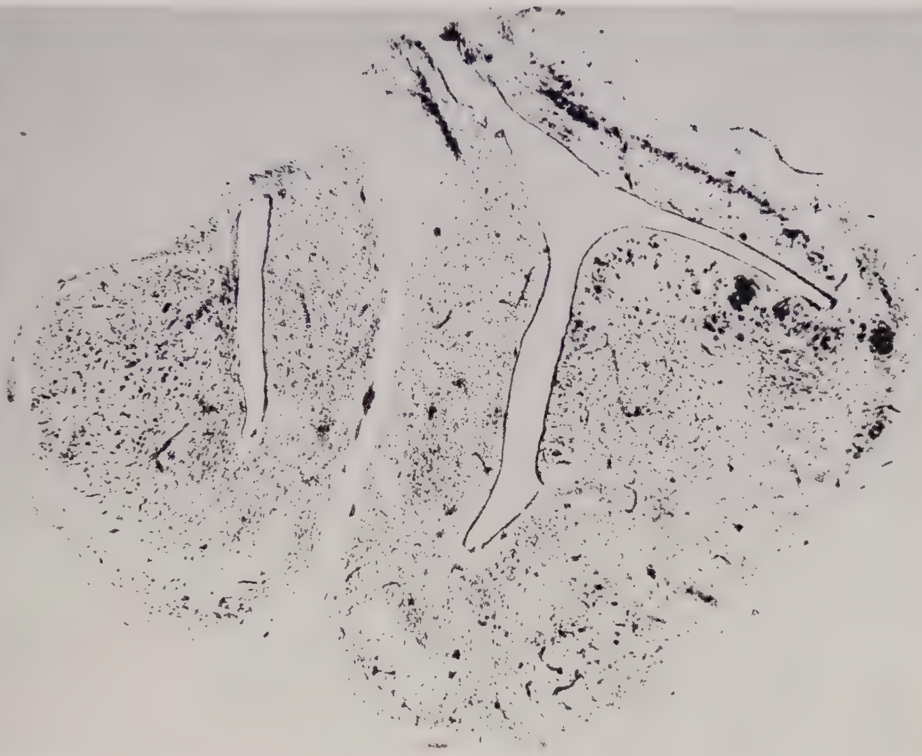
Fig. 5. Photomicrograph to show the retrograde cell degeneration in Expt. OL 65. Stained with thionine. × 93.

Fig. 6. Photomicrograph to show severe fibre loss in the medial and lateral forebrain bundles at the level of the preoptic areas in the same experiment as the preceding figure. Bodian's protargol stain. × 72.

Plate 4

Fig. 7. Photomicrograph showing degeneration of the anterior thalamic component of the lateral forebrain bundle on the right side of the same experiment. Bodian's protargol method. × 65.

Fig. 8. Photomicrograph to show atrophy of the intermediate thalamic component of the lateral forebrain bundle on the right side of the same experiment. Bodian's method. × 80.



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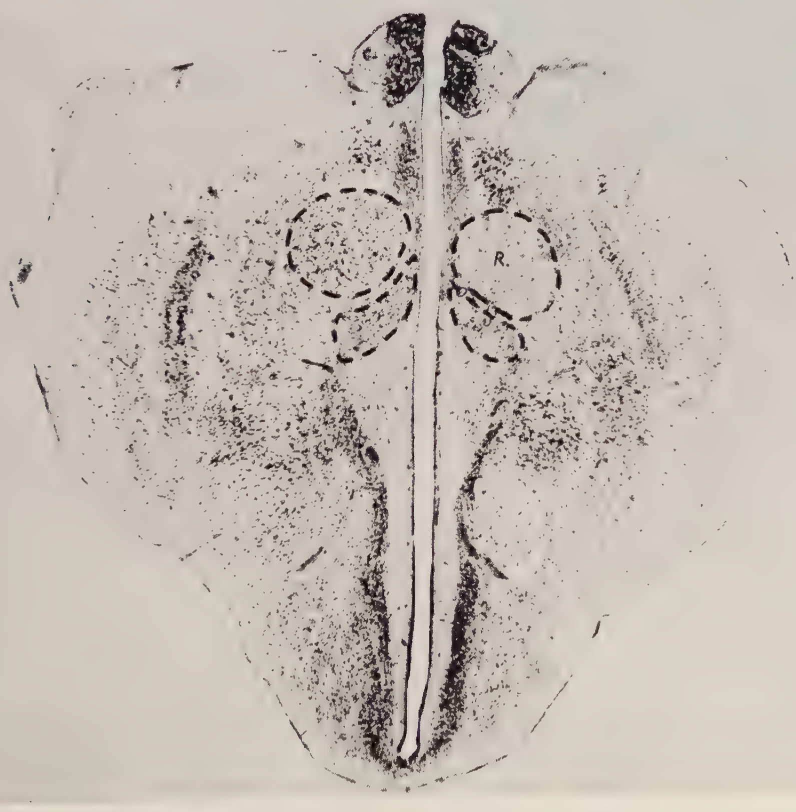

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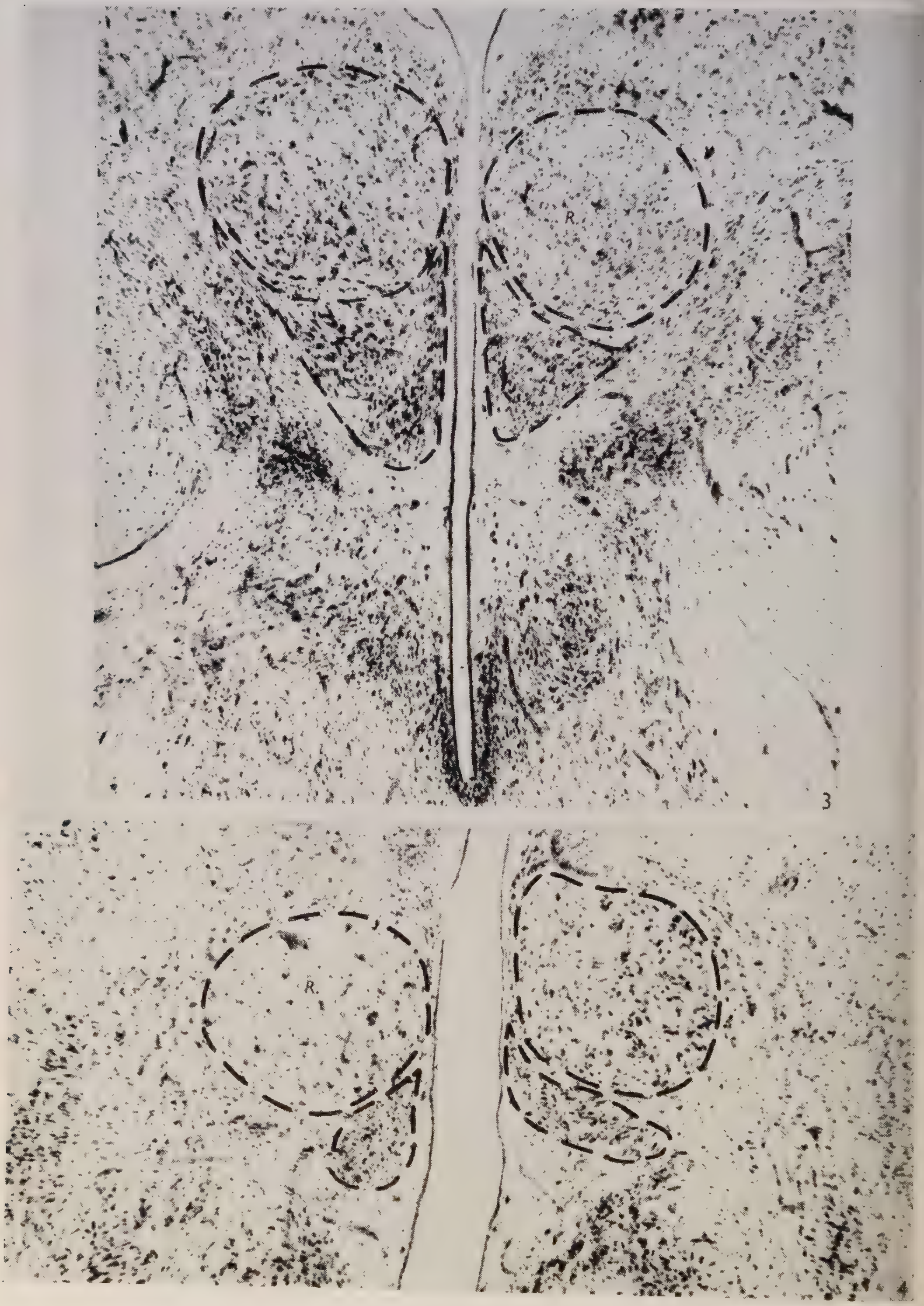
POWELL AND KRUGER. THALAMIC PROJECTION UPON THE TELENCEPHALON IN *LACERTA VIRIDIS*



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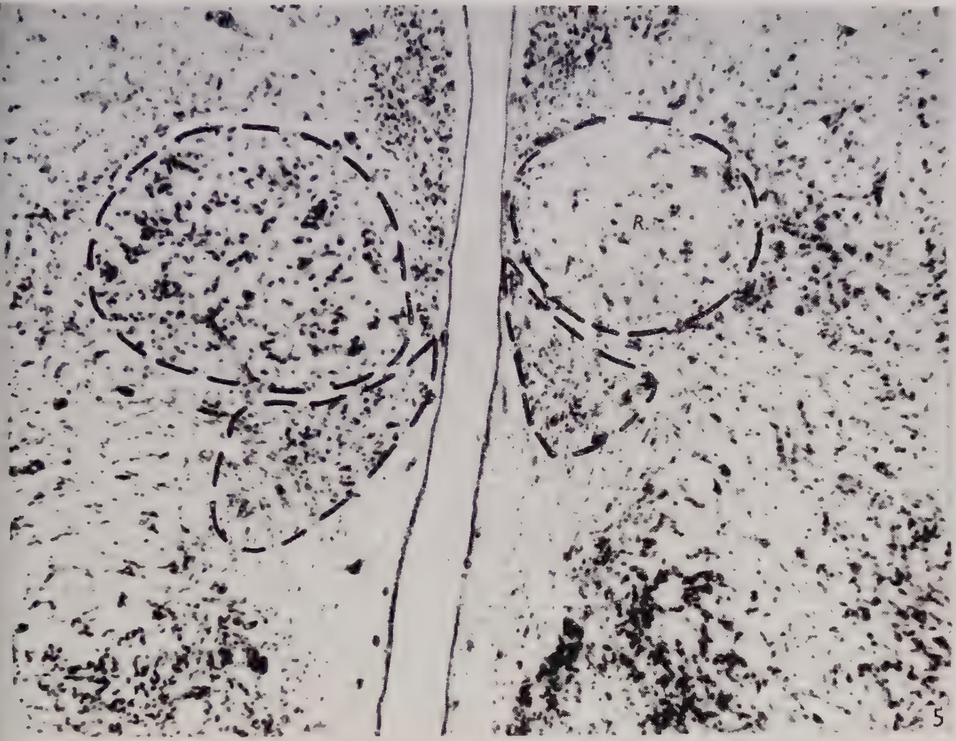

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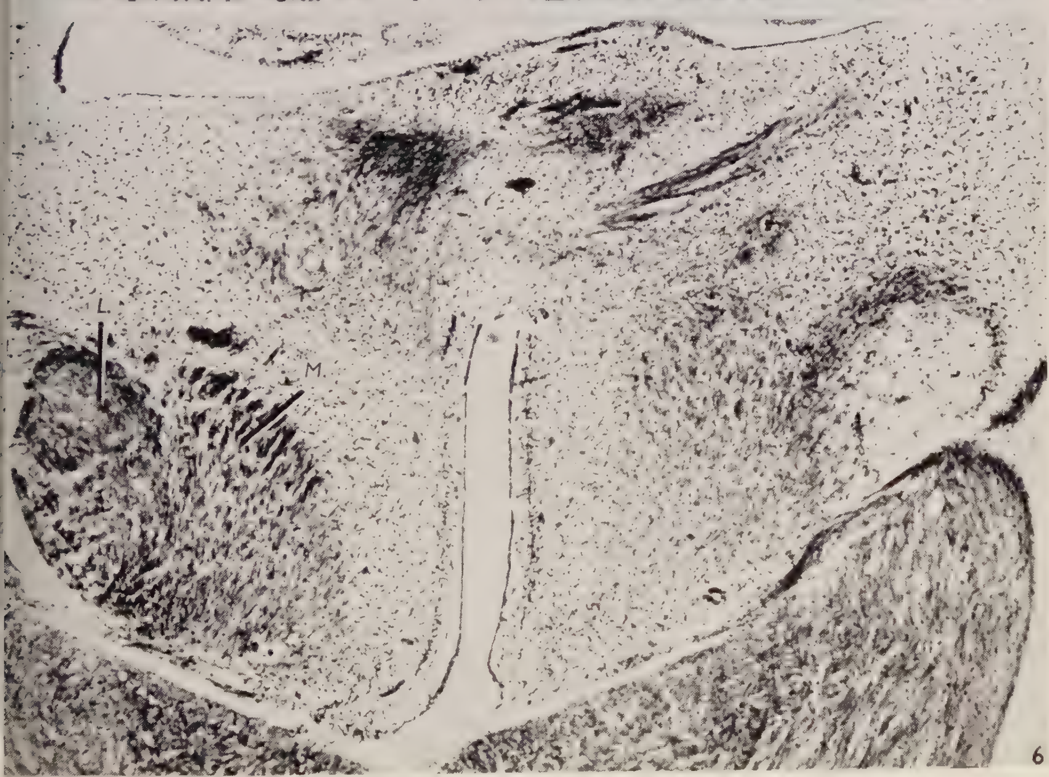

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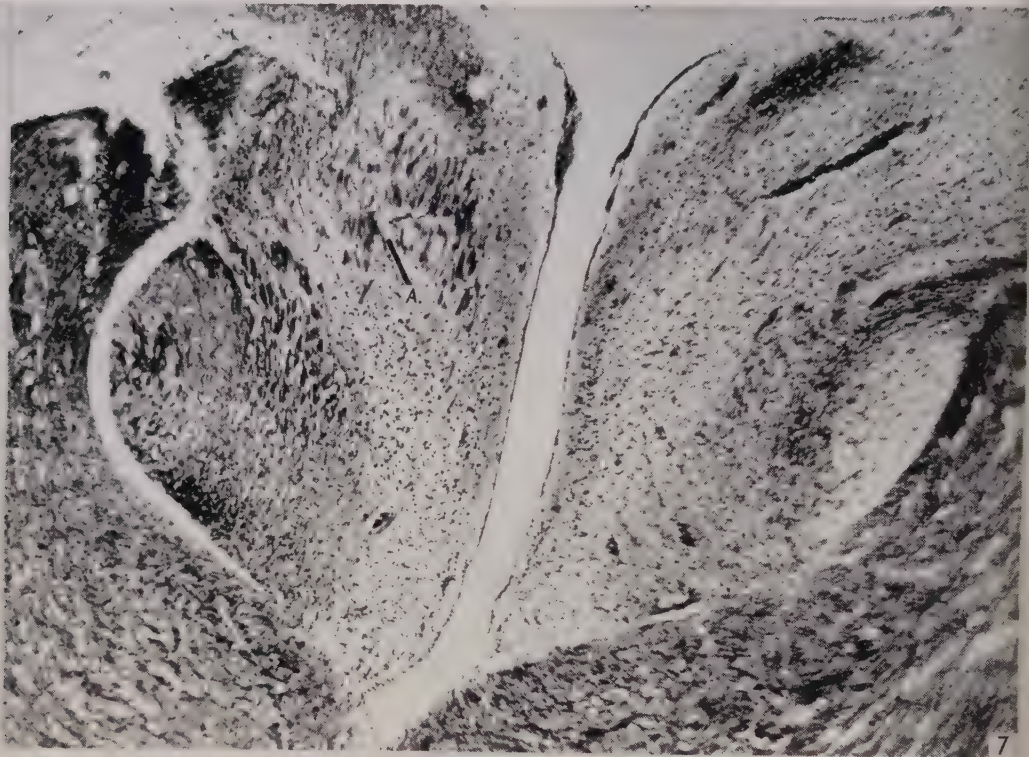

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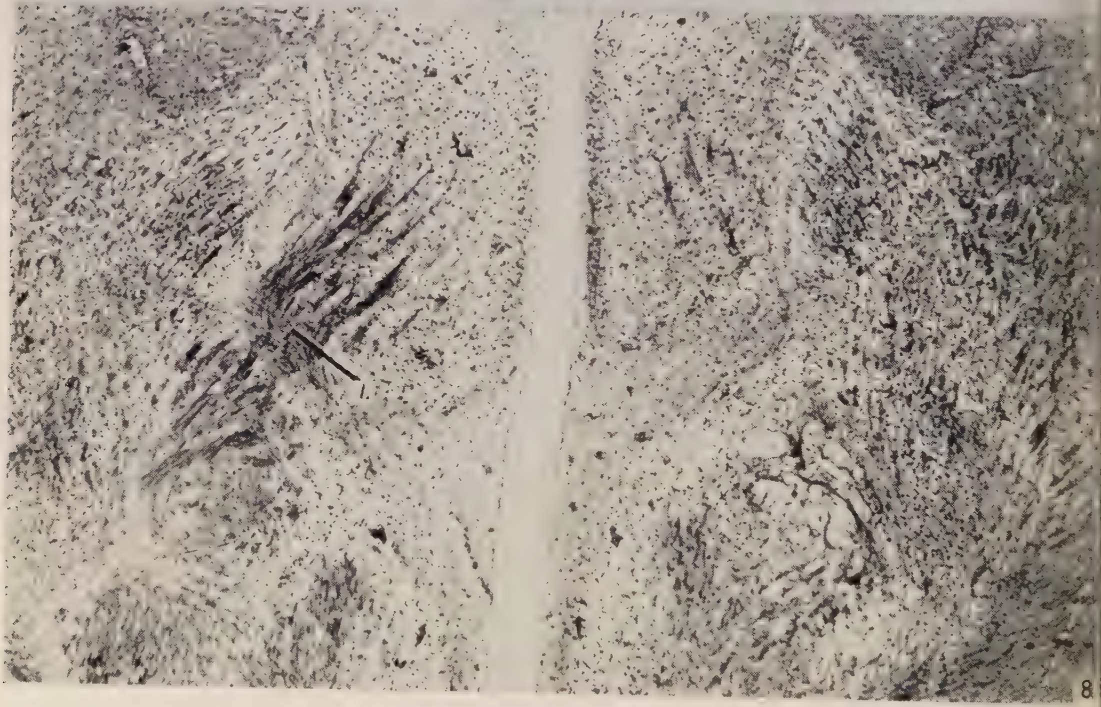

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POWELL AND KRUGER. THALAMIC PROJECTION UPON THE TELENCEPHALON IN *LACERTA VIRIDIS*

SCROTAL ASYMMETRY AND HANDEDNESS

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INTRODUCTION

The asymmetry of the human scrotum is an intriguing morphological phenomenon, for which diverse views and explanations have been presented. Current standard English text-books of human anatomy (Gray, Cunningham, Morris) describe the left side of the scrotum as descending lower than the right to correspond with the lower position of the left testis or with the longer spermatic cord. This statement is only partially true, for while testicular position and scrotal form are certainly correlated, the left side of the scrotum is not always the lower. Furthermore, the statement is ambiguous in that it is sometimes assumed to be based on an embryological explanation, i.e. that the left testis descends in advance of the right, or that the left spermatic cord is longer because of the association of the left testis with the more cranially situated left kidney. For these claims we can find no substantiation in standard embryological treatises.

Poirier & Charpy (1923) state that the testes of infants at the time of birth are situated at the same level in the scrotum, but later the left testis becomes lower than the right. They go on to say (and we give a translation of their account): ‘One admits in general with Wincklemann (an observation well shown by sculptors before him) that the left testis is situated usually $\frac{1}{4}$–1 cm. lower than the right. Some anatomists, Charpy, for example, contend this view. One may rightly ask why the testes, which are situated at the same level in infants, should occupy unequal levels in the adult? One might wish to explain the lower descent of the left testis as due to a congenital inferiority of the left side. It would be equally wrong to assume that this lower position is due to the heavier weight of the left testis, a fact which has not been demonstrated. It appears that, with Poirier, one should ascribe the cause to the very position of these glands in the angular interval between the thighs after leaving the neighbourhood of the pubis. Situated in this space, the testes, overlapping and pressing on each other, would adapt themselves by gradually modifying their positions. Suspended by the spermatic cords, the testes are mobile in all directions which permit them to escape shocks; they ascend towards the external inguinal ring by the contraction of the cremaster, and descend by their weight.’ This teleological account, however, leaves unanswered the question why it is that the left testis is generally lower. If it were merely a matter of adaptation of the testes to the limited wedge-shaped space between the thighs, then the chances of either testis to occupy a higher or lower position would be theoretically about equal.

Braus (edited by Elze, 1956) approaches the problem from an entirely different angle, that of the venous drainage of the testes by the spermatic veins, i.e. the right vein joining the inferior vena cava at an acute angle and the left joining the left renal vein at right angles, with the accompanying disadvantage of increasing the resistance to the venous return from the left side. He states that on account of this increased resistance and a consequent vascular stasis in the tributaries of the left spermatic vein, the left testis is heavier and normally hangs slightly lower than the right. According to our findings to be stated later, the right testis is in fact generally heavier and bulkier than the left.

MATERIAL AND METHODS

(1) *The subjects and observation of the scrotum.* The subjects for examination were all Chinese, drawn from three unselected groups, namely (1) hospital orderlies (95), (2) general out-patients (304) and (3) students of Hong Kong Sea School (87), totalling 486. The ages of these subjects ranged from 14 to 64, and the distribution is shown in Table 1, with the number of subjects arranged under the columns of right- and left-handers. Subjects having abnormalities of any kind in the scrotum such as hydrocoele, pathological conditions of the testis or epididymis, agenesis of testis or any sign of dermatitis of the scrotal skin, were excluded. The examinations of the first two groups were performed during the hot summer months, and those of the last group in the autumn with the room temperature of 70–75° F. They were unaware of the reason for the examinations, accepting them as part of a routine 'check-up' in health.

Table 1. *Subjects examined* (486)

	Number of subjects	
Age	Right-handers	Left-handers
14	22	—
15	13	2
16	18	2
17	15	—
18	21	1
19	4	1
20–24	62	10
25–29	71	6
30–34	72	8
35–39	51	—
40–44	43	3
45–49	26	—
50–54	17	1
55–59	10	—
60–64	6	1
Total	451	35

To ensure as complete relaxation as possible, each subject was engaged in informal conversation throughout the examination. He was asked to stand facing the observer with his feet 18 in. apart, and a spirit level ruler, marked with a millimetre scale and adjustable in height and level, was placed standing below the scrotum to serve as a gauge for measuring the relative level of the two sides of each subject. The levels were noted on a card, together with the name and age of each subject. To test the effect of exertion on the position of the testes and of the scrotum, a weight (25 lb. for subjects older than 20, and 10 lb. for subjects younger than 20) was passed to his right hand first, to be lifted vertically close to the right side of the body. The weight was then transferred after a short interval to the left hand and lifted on that side; this order was maintained throughout the tests of all the subjects and the effects on scrotal levels were, in each case, recorded on the card. In many cases these tests were repeated and the contraction of the abdominal muscles demonstrated by palpation. Photographic records were made of the scrotum in all the stages of the examination.

Only simple tests for handedness were made, which consisted of throwing, reaching and kicking three times in different order. Though crude, they provided adequate evidence of functional dominance for our present purpose. Preference of the hand and foot used in these tests, and questioning the subjects on their preference in other activities were taken as criteria of handedness.

(2) *The weight, volume and position of the testes.* The scrota of 100 cadavers were examined in the mortuary, a record was kept of the age and the relative level of the two sides of the scrotum, and a compass measurement was made of the distance from the lower pole of each testis to the mid-point of the pubic arch. The tunica vaginalis was shelled out of the scrotal sac and removed with the spermatic cord and labelled. A slit was made in each tunica to see the upper border of the epididymis, at which level the tunica and the spermatic cord were cut across, and the post-mortem exudate, if any, evacuated. The tunica with its contents and the associated portion of the spermatic cord were weighed together. Finally, each testis was dissected free of the tunica, epididymis and other extraneous tissue and weighed. The greatest circumference of the right and left forearms was measured as indication of handedness. Specimens showing abnormal or diseased conditions were excluded.

OBSERVATIONS

(1) *Relative level of the two sides of the scrotum in living subjects.* The subjects numbered 486, of whom 35 were left-handed. The percentage difference in the relative level of the two sides of the scrotum is opposite in the right- and left-handers, the right-handers having the right side higher in 64·7 %, and the left-handers having the left side higher in 68·5 % of cases. The exertion tests definitely affected the position of the testes by raising them, and the characteristics of the effects can be divided generally into two categories: (1) In the majority of cases the effect was seen to be more on the testis of the same side and relatively less, though present, on the testis of the opposite side but on the whole not to the extent of altering the relative level seen before testing, except in fourteen cases. In these latter cases the original relative level was altered depending on the side exerted, that is to say, when the right side was exerted, the right testis went up to a relatively higher level than the left and vice versa. (2) In a minority of cases the effect of exertion was on the testis of the opposite side. In twenty-three cases, right and left exertions raised the testis of the opposite side higher than the original level before exertion, and in ten cases the position of the testis was variable depending on the side exerted, i.e. when the right was exerted, the left testis went up higher than the right testis and vice versa when the left was exerted.

The χ^2 test ($P = < 0{\cdot}01$) shows a significant correlation between scrotal asymmetry and handedness (both without and with exertion). Our findings are at variance with those of Antliff & Shampo (1959).

(2) *The relative position, weight and volume of the right and left testes obtained from cadavers in the mortuary.* Although measurements of the forearms were taken and

by these measurements five of the cadavers were assumed to be definitely left-handers, a few were borderline cases about which we could not be certain. It has been considered best, therefore, to consider all the cases together without regard to handedness. The right testis was found to be higher in 65 % of cases, the left testis higher in 33 %, and both testes occupying the same level in 2 % of cases. Table 2 gives our data of the relation of the weight of the testes to their position. Table 3 gives the mean weight of the right and left testes with and without adnexa (i.e. tunica vaginalis, epididymis and the associated part of the spermatic cord), mean volume, mean difference and probability.

Table 2. *Relation of testicular weight to position in cadavers*

	Incidence of occurrence	
	Right testes	Left testes
Testes higher and heavier	42	5
Testes lower and heavier	27	20
Testes heavier and occupy same level	2	0
Testes of same weight but higher on one side	3	1
Testes higher and lighter	20	27
Testes lower and lighter	5	42
Testes lighter and occupy same level	0	2
Testes of same weight but lower on one side	1	3
Total	100	100

Table 3. *Mean weight and volume of right and left testes*

		Mean weight of testes (g.)		Mean volume of testes (c.c.)		Mean weight of testes with adnexa (g.)	
Age	N. (100)	Right	Left	Right	Left	Right	Left
10–19	1	12·90 ± 0	13·10 ± 0	12·50 ± 0	13·00 ± 0	17·90 ± 0	19·00 ± 0
20–29	3	12·44 ± 2·33	11·53 ± 1·64	12·20 ± 2·29	10·90 ± 1·35	20·05 ± 0·14	17·85 ± 0·14
30–39	25	10·89 ± 0·59	9·94 ± 0·64	10·64 ± 0·59	9·65 ± 0·64	17·58 ± 1·07	16·72 ± 1·16
40–49	37	9·95 ± 0·54	9·60 ± 0·48	9·63 ± 0·53	9·35 ± 0·47	16·24 ± 0·85	15·54 ± 076
50–59	18	9·57 ± 0·78	8·95 ± 0·62	9·40 ± 0·76	8·73 ± 0·59	15·51 ± 1·21	14·43 ± 1·03
60–69	10	8·81 ± 1·27	7·87 ± 1·17	8·58 ± 1·25	7·62 ± 1·16	17·57 ± 2·19	17·18 ± 2·12
70–79	6	7·32 ± 0·67	7·47 ± 1·20	7·05 ± 0·64	7·17 ± 1·20	14·57 ± 0·90	13·95 ± 1·17
Mean total		9·95 ± 0·33	9·36 ± 0·31	9·69 ± 0·33	9·10 ± 0·30	16·60 ± 0·52	15·81 ± 0·50
Mean difference		0·59 + 0·09 ($P = < 0·001$)		0·59 + 0·09 ($P = < 0·001$)		0·79 + 0·26 ($P = < 0·01$)	

DISCUSSION

We consider the subjects reported herein a reasonably reliable sample, judging by the essentially similar results we obtained from all of the three groups, taken either separately or together. We are of the opinion that three conditions are requisite for obtaining reliable results, namely, unawareness of the purpose of the examination on the part of the subjects, warm and even temperature, and relaxation. We have purposely avoided the use of medical students as subjects because of the difficulty experienced in excluding psychological factors.

It has generally been taken for granted that the scrotum is asymmetrical with the left side occupying a lower level than the right. The finding among our series of 486 subjects shows instead an incidence of 10·5 % of scrota with the two sides occupying the same level, 62·1% with the right side higher than the left and 27·4% showing the left side higher than the right. Furthermore, our data show the right side of the scrotum higher than the left in about 64·7% of right-handers, and nearly the reverse for left-handers, i.e. the left side higher than the right in 68%. These latter figures indicate clearly a correlation of scrotal asymmetry with handedness, which in itself may be considered as a conspicuous expression of a more general dynamic 'laterality' of the body. Perhaps the relative level of the two sides of the scrotum may prove to be a crude index to the 'laterality' of the trunk.

For our consideration of the scrotum from the angle of dynamic 'laterality', it may be helpful to free our thoughts from the trammel of embryological details and to look upon the scrotum simply as the lower paramedial parts of the ventral abdominal wall drooping down, i.e. the whole thickness of the ventral body wall 'dew-lapping' between the thighs, and to look upon the testis as extruded viscera and the saccus vaginalis as a lesser part of the general abdominal cavity isolated from the latter. The smooth musculature or the dartos of the scrotal skin is continuous with the peri-penile and peri-anal smooth musculature, all of which have spread out from the urogenital and anal triangles of the primitive perineum. The striated musculature of the scrotum is continuous with, in fact part and parcel of, the musculature of the ventral abdominal wall, but due to a functional modification the aponeurosis of the external oblique is here reduced to form the filmy external spermatic fascia, while the internal oblique mainly, and the transversus partly, form the cremaster whose function is to protect and sustain the underlying viscera in a manner comparable with the rest of the ventral abdominal muscles which overlie the viscera of the general abdominal cavity. The cremaster is known to draw the testes up for protection and it is interesting to mention in this connexion that one of the first lessons in Chinese methods of boxing is to train the cremaster to withdraw the testes from harm. Among other functions, the ventral abdominal muscles take part in postural reactions involving the flexion and rotation of the trunk as well as in the fixation of the ribs, sternum and pelvis in all the more vigorous movements of the limbs. The internal oblique of one side, which is continuous with the cremaster, flexes and rotates the trunk to the same side, an orientation which the body tends to assume as the hand approaches an object or a task. It is not unreasonable, therefore, to conjecture that the limb most employed, which is the right limb for most of us, tends to heighten the activities of the internal oblique and the cremaster of the same side.

The 30–35% of exceptions to our concept of correlation of scrotal level with handedness are important and they deserve careful study by electromyographic methods, such as used by Floyd & Silver (1950) in their study of the patterns of activity in the abdominal wall muscles in man. However, these discrepancies are to be expected for we are dealing with patterns of variable factors. Handedness in itself is an extremely complex trait; it can be strongly, mildly or weakly dominant; it can be mixed or covered. Also, posture of the body in greater or lesser measure must have its bearing on the activities of the internal oblique and cremaster both unilaterally and bilaterally.

Certain interesting facts emerge from an analysis of our data of testicular weight and its relation to position (Table 2), which would seem to shed some additional light on the problem: (1) the χ^2 test shows that the right testis is generally heavier than the left ($P = < 0{\cdot}001$). This would argue strongly against the thesis sometimes held that the left testis is usually lower than the right because of its heavier weight; (2) there would appear to be no association between testicular weight and position (higher or lower), according to the χ^2 test ($P = < 0{\cdot}3$). It may be noted from Table 2 that of the right testes that are higher the majority are heavier, whereas with the left testes the reverse obtains. If weight is not a factor in influencing testicular position, then there must be some other factor or factors. It appears to us highly probable that the differential tonus or activity of the cremaster is an important factor.

If our concept of the cremaster as a contributory factor to scrotal asymmetry proves to be significant, it will be interesting to see what relationships it may have with certain other problems which concern the scrotum and testes. Cholst (1947) reported six cases of *situs inversus totalis*, all of whom had the right testis lower than the left. In fact, he considers this testicular position as typical of *situs inversus totalis*, his explanation being venous transposition. It is important, however, to know whether these cases also showed transposed functional 'laterality'. With regard to varicocele which happens generally on the left side, a weaker cremasteric tonus may be one of the predisposing factors aside from venous arrangement.

SUMMARY

The scrota of 486 subjects were examined. Exertion tests were applied which showed their effects on the position of the testis. The relative level of the two sides of the scrotum, without or with exertion, shows a correlation with handedness. The weight, volume and position of the testes were studied from 100 cadavers. It is shown that in the majority of cases the right testis is heavier than the left and that there seems to be no association between testicular weight and position.

Our sincere thanks are due to Professor Sir Wilfrid Le Gros Clark for his helpful suggestions. We would also like to express our thanks to Dr K. C. Tsang, Sister M. Aquinas, Dr A. R. Hodgson, Dr S. Bard, Dr Peter Wong, Dr Raymond Lee, Dr Doris Gray, Dr Arnold Hsieh, Rev. E. Kvan, Mr Brook Bernacchi and the staff of Hong Kong Sea School, Mr Lee Shing and Mrs Emily Hsieh for their kind assistance.

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THE MEDIAN APERTURE OF THE FOURTH VENTRICLE

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The existence of the median aperture of the fourth ventricle in the human brain is generally accepted today. The majority of the standard text-books describe it as a hole in the lower part of the roof of the fourth ventricle facing backwards and somewhat downwards with the vertical limbs of the chorioid plexus extending to its upper margin. A certain number of these descriptions add that the vertical limbs of the chorioid plexus protrude through the aperture in a manner similar to that in which the horizontal limbs of the plexus protrude through the lateral apertures of the ventricle. In text-books illustrating the region of the median aperture difficulty is experienced because the text description frequently does not agree with features shown in the illustration. Many of these illustrations are of median sections through the brain stem and cerebellum showing the upper border of the aperture to be ill defined and the vertical limb of the chorioid plexus curving downwards and backwards along the side of the tonsil of the cerebellum. In some books illustrating the median aperture from below, the upper part of the aperture is prolonged into a triangular fold over the anterior end of the inferior vermis of the cerebellum and containing part of the vertical limb of the chorioid plexus. Only two of these text-books (Brash, 1958; Mettler, 1948), contain any reference to this fold in the text. The configuration and dimensions of the aperture were examined by Barr (1948). Although varying markedly in size the aperture was present in all but one of 118 human brains, and he also found this fold present in some form or another in a large percentage of the specimens. His description is not easily followed but in spite of this it is strange that this feature, probably well known to many teachers of neuroanatomy, has not yet been incorporated into text-book descriptions. Although this may be regarded by some as of purely academic interest it may well have a more practical clinical significance, as Barr points out. The observations described here provide some measure of support for those of Barr and it is hoped that by reporting them attention will be drawn to these facts enabling the form of the aperture to be better understood.

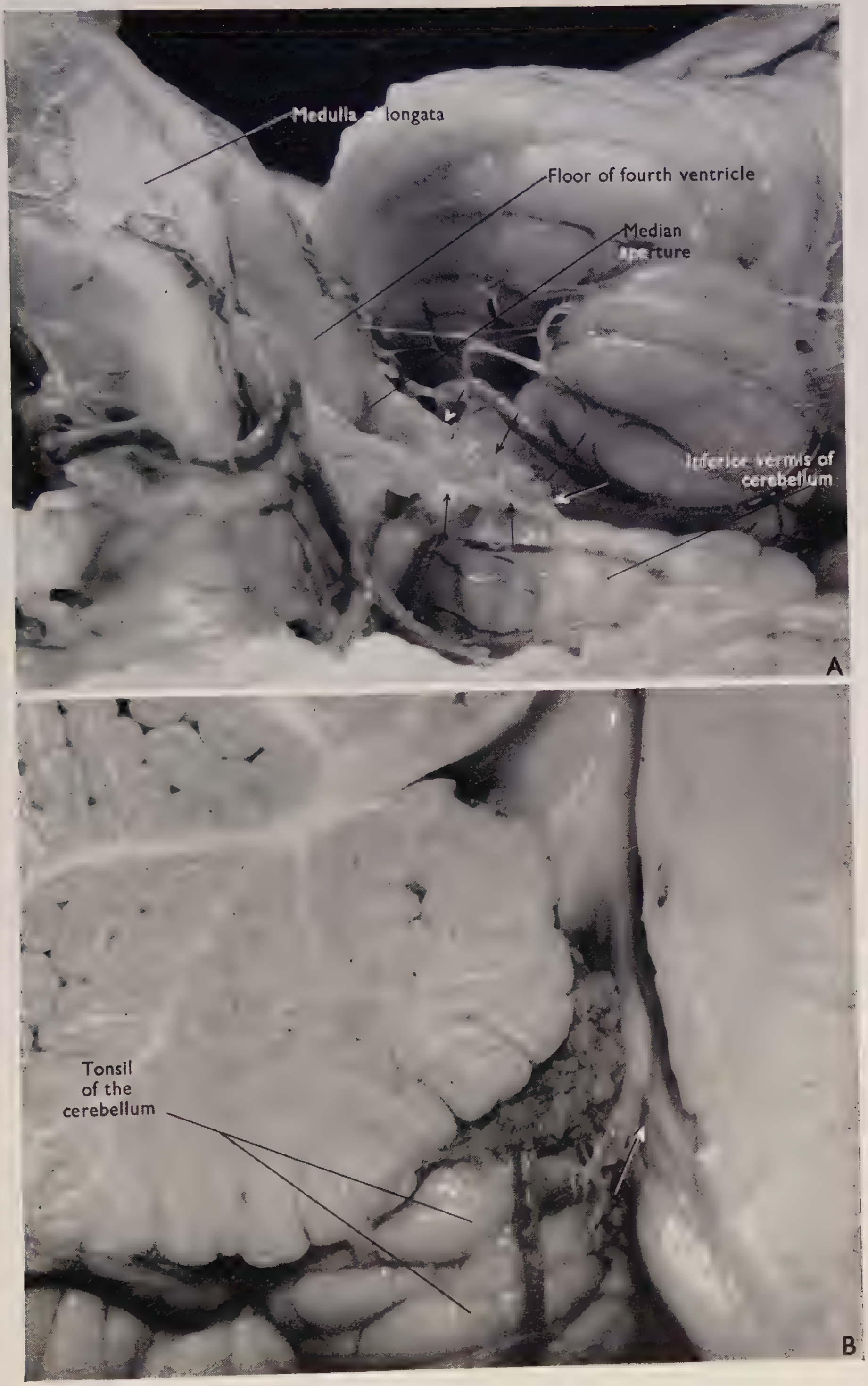
Thirty-eight, formalin fixed, adult human brains were specially examined; the principal feature of the aperture described here has, however, also been frequently seen in many fresh brains and for a number of years in brains used in classes dissecting the human brain. The median aperture was displayed with the minimum of disturbance by dividing, when necessary, the arachnoid between the cerebellar hemispheres. Usually after this the median aperture was clearly visible but sometimes it was partially obscured by a variable number of trabeculae in the subarachnoid space. In all specimens the foramen was present. In thirty specimens the pia-ependymal roof of the ventricle was prolonged backwards at the upper part of the aperture as a triangular fold adherent in some degree to the pia on the under aspect of the anterior end of the inferior vermis (Pl. 1, A). In most cases the base of the fold was unattached to the vermis and a space could be seen between them (Pl. 1, A). In all cases the fold could easily be freed from the inferior vermis and if it was then allowed to flap down over the median aperture this assumed the shape of a V-shaped slit. The attachment of this fold to the vermis resulted in a tent-shaped appearance to the roof of the ventricle above the aperture which, as a result, was somewhat funnel-shaped and directed downwards and slightly backwards with an ill-defined upper boundary. This funnelling of the aperture was accentuated when the tension was increased by drawing forwards the medulla oblongata as in Pl. 1, A. This fold, usually about 1 cm in length, varied from its almost complete absence to one instance in which it extended more than halfway along the inferior vermis. Contained in the edges of the fold were the lower ends of the vertical limbs of the chorioid plexus of the ventricle and these came into contact or fused at the apex where they terminated, except in two brains when the united plexus continued beyond the apex as a single plexus attached to the under surface of the inferior vermis. Thus, the lower part of the vertical limbs of the plexus extended beyond the limits of the aperture and lay in the upper part of the cerebello-medullary cistern. In some brains a median section of the brain stem and cerebellum was made, dividing the triangular fold between the vertical limbs of the chorioid plexus and each plexus was then seen curving downwards and backwards on the surface of the tonsil of the cerebellum of its own side (Pl. 1, B).

In eight brains the triangular fold was absent and in these the vertical limbs of the chorioid plexus extended separately beyond the aperture, each being adherent to the pia mater over the tonsils of the cerebellum of its own side. The absence of this fold could, however, have been an artefact resulting from the splitting of the triangular fold as the cerebellar hemispheres were separated to obtain a clearer view, for in many of those with a fold present this was often so thin it could have been torn by careless handling.

The form of this region may be clarified if it is likened to the effect produced by pulling on the tongue of a toeless slipper, the tongue being equivalent to the triangular fold. The cavity of the slipper represents the lower part of the ventricular cavity continuous with the remainder of the cavity through the toe, the sole being the floor and the upper the roof. The opening of the slipper represents the median aperture and when the tongue is pulled away from the floor the opening is converted into a funnel into which the foot can more easily slide.

SUMMARY

In thirty-eight human brains the median aperture of the fourth ventricle has been examined. In thirty brains the roof of the ventricle was prolonged at the upper part of the aperture as a triangular fold containing the lower part of the chorioid plexus and attached to the pia over the anterior end of the inferior vermis. In eight brains the fold was absent and in these the vertical limbs of the chorioid plexus were continued beyond the margin of the aperture and were adherent to the pia over the tonsil of the cerebellum. When present the triangular fold converted the median aperture into a funnel-shaped opening.



HEWITT—THE MEDIAN APERTURE OF THE FOURTH VENTRICLE

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EXPLANATION OF PLATE

A. View of the median aperture in an inverted human brain in which the medulla oblongata has been pulled forwards. The triangular fold containing the chorioid plexus extends backwards from the median aperture and is outlined by the arrows. Near its apex the fold is adherent to the pia mater over the inferior vermis of the cerebellum. × 4.

B. Median section of the human brain stem and cerebellum. One half of the chorioid plexus and triangular fold can be seen curving downwards and backwards over the surface of the tonsil of the cerebellum. The arrow indicates the site of the funnel-shaped median aperture. × 4.

SEQUELAE OF EXPERIMENTAL PARTIAL ISCHAEMIA IN LONG BONES OF THE RABBIT

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Previous studies on experimental ischaemia of bone have tended to be restricted to an examination of ensuing cellular changes principally in the bone marrow, the question of the direction of blood flow in ischaemic bone being neglected. Differences of opinion exist as to the nature and degree of these cellular changes (Houang, 1934; Kistler, 1934; Higgins & Wiege, 1939; Bragdon, Foster & Sosman, 1949), and in some investigations the procedures employed go beyond the production of simple ischaemia, introducing uncertainties in the interpretation of the results (charcoal embolism, Kistler, 1934; marrow destruction, Marneffe, 1951; periosteal stripping, Foster, Kelly & Watts, 1951). For these reasons, it was decided that the effects of uncomplicated ischaemia on bone should be studied anew, with special reference to the circulatory pathway in the compactum as well as to the cellular reactions in the cortex and marrow.

MATERIALS AND METHODS

Sixteen adult rabbits were used in the course of this investigation. In each the arterial supply to the right femur and tibiofibula was partially suppressed by ligation and section of the principal nutrient artery. To lay a heavier ischaemic stress on the tibiofibula, the popliteal artery was sectioned between ligatures in the adductor canal below the origins of the A. suprema genu and the saphenous artery. The former provides a large anastomotic pathway to the leg and the latter, by means of its medial and lateral plantar divisions, the bulk of the blood supply to the rabbit foot. Hence the ensuing ischaemia predominantly affected the two bones in question and peripheral gangrene did not occur. The left limbs were used as controls.

Three rabbits were killed at weekly post-operative intervals for 1 month and thereafter one rabbit at monthly intervals of 5 months. Before killing, under ether anaesthesia, the arterial system was filled with a suspension of barium sulphate (Micropaque, Damancy and Co.) injected through a polythene cannula tied into the abdominal aorta. Micropaque was diluted to half strength with normal saline and injected at a manometrically-controlled pressure of 70–100 mm. Hg until complete filling of the arterial system of the posterior extremities was judged to have taken place, utilizing the coincidental visible filling of the gut wall vessels as an indicator. After injection with Micropaque the rabbit was killed by ether overdosage and the hindlimbs and pelvis skinned and fixed in one piece in a 5 % formalin (2 % formaldehyde) solution. To avoid loss of injection medium before fixation, care was taken to ligate vessels oozing Micropaque from cut surfaces. Two weeks were generally allowed for fixation before examining the parts radiologically and histologically. Some specimens were radiographed whole immediately after fixation in order to compare the radiological appearances of the bones and arterial tree in the experimental and control limbs. Soft tissues were then removed until only the periosteum and immediately adjacent and attached muscle fasciculi remained about the femora and tibiofibulae; these specimens were decalcified in a 5 % nitric acid in 5 % formalin solution. Transverse sections about 1 mm. thick were cut by hand from the upper, middle and lower thirds of each bone, and these, together with other longitudinal sections, were microradiographed using Kodak maximum resolution plates and a microfocus unit incorporating the Ehrenberg and Spear tube. Other specimens were blocked in paraffin wax using Peterfi's (1921) double embedding technique, and sectioned transversely and longitudinally at 8 μ. Sections were stained with Ehrlich's haematoxylin and eosin and Mallory's trichrome stain. A few representative sections from operated and control limbs were also stained by Wilder's (1935) silver method.

RESULTS

Radiological observations

Whole plate views of the rabbit hind quarters taken prior to decalcification showed differences between control and operated sides both in the radiotranslucency of the bones and in the soft-tissue arterial pattern. After 4 weeks of ischaemia the bones of the operated limbs were less opaque to X-rays than their controls, the increase in radiotranslucency becoming more marked in succeeding months. On the other hand, the density of small arterial channels in the soft tissues of the ischaemic leg increased during the same post-operative period and at 5 months was in marked contrast to the arterial pattern of the contralateral limb, presenting as a cloudy haze of fine vessels below the level of section of the popliteal artery against the more discrete pattern in the same region of the control (Pl. 1, fig. 1).

Microradiography of thin sections cut from the control femora and tibiofibulae showed the general vascular features of the cortex and medulla of long bones. The sharply defined arterial pathways of the medulla were more consistently represented in control microradiographs than were the sinusoids of the marrow, which in some sections were hardly visualized peripherally beyond the central venous sinus. Medullary arteries tended to course radially towards the cortex (Pl. 1, figs. 3, 5) when seen in cross-sections of bone, while in longitudinal sections main arteries were found running in the periphery of the medulla giving off fine terminal branches which pierced the endosteal cortex and passed centrifugally towards the periosteum, being traceable usually as far as the outer half of the compactum (Pl. 2, fig. 7). Although periosteal and surrounding soft tissue vessels were readily visualized by radiography following Micropaque injection, control microradiography did not demonstrate anywhere a centripetal penetration of barium sulphate suspension into the underlying bone cortex.

Microradiography of transverse and longitudinal sections cut from the ischaemic limbs and leaving soft tissue adjacent to bone *in situ*, showed that in both femur and tibiofibula functioning vascular structures were still present in cortex and marrow. Marrow sinusoids opened in dense groupings either directly into the central venous sinus of the medulla or into its large radially disposed tributaries. Here and there the appearances were suggestive of a lobular organization of the marrow (Pl. 2, fig. 8), patches of sinusoids rendered radiopaque by intravital injection of Micropaque being separated by zones not reached by the injection mass. When sinusoidal filling was virtually complete, the patchy lobular pattern of groups of sinusoids each draining into their respective tributary of the central venous sinus was not seen, but instead the sinusoids formed a uniform reticular background against which discrete arterial channels could be discerned (Pl. 2, fig. 9). Nevertheless, in the diaphyseal marrow of the ischaemic femur and tibia, sharply defined areas of densely packed and filled medullary sinusoids adjoined patches of radiologically empty medulla (Pl. 2, fig. 10). Such areas of medullary infarction were demonstrable by radiography up to 1 month post-operatively.

In the cortex of the ischaemic femur, the same centrifugal pattern of arterialization as in the controls was found at first, notwithstanding the dense vascular filling of attached musculature; but from 4 weeks post-operatively there was microradiographic evidence of the centripetal passage of barium sulphate suspension from the periosteal circulation into the underlying bone (Pl. 1, figs. 4, 5). In the cortex of the ischaemic tibia haemodynamic changes were more pronounced and were observed earlier. Two weeks after the vascular ligations Micropaque passed unmistakably from the surrounding soft tissue vessels centripetally into the cortex (Pl. 2, fig. 10). At first the periosteal arterial pathways in the compact cortical bone were fine in calibre and penetrated only the outer half of its thickness, being especially numerous opposite areas of medullary infarction; but with an increasing post-operative interval the periosteal arterial supply of the cortex became more evident (Pl. 1, figs. 2, 4), and there were numerous large anastomotic vessels passing between the periosteal and medullary arteries (Pl. 2, fig. 6), while other centripetal arteries passing through the cortex opened directly into the medullary sinusoids (Pl. 2, fig. 11). These dramatic changes in the pattern of bone vascularization affected predominantly the upper two-thirds of the tibial shaft: by the end of the second post-operative month they were fully developed and persisted thereafter, without diminution, for a further 3 months at least.

Histological observations

One week after the arterial ligations the marrow of the affected long bones showed widespread changes. These did not, however, involve the whole thickness of the medulla, some normal haemopoietic tissue always being present (Pl. 3, fig. 13). Adjacent to infarcted areas, where the cells had lost their differential staining properties and the sinusoids were collapsed, there were zones where some of the sinusoids were still functional as shown by the presence in them of injected barium sulphate granules as well as the presence around them of normally staining marrow cells. Some spicules of new bone had formed as projections from the endosteal surface of the cortex. The cortex itself was little affected at this early stage: occasionally some enlargement of the vascular canals was observed (Pl. 4, fig. 20), but usually the canals and cells of the compactum of both femur and tibia differ little from the normal controls. In particular the osteocytes of recently ischaemic compact bone did not undergo extensive necrosis; only in the intermediate zone, and there generally confined to the interstitial lamellae, were there found isolated osteocytes showing poor nuclear staining and occasional empty bone lacunae. Such appearances occurred only in scattered fields and then uncommonly.

From 2 to 4 weeks post-operatively the reaction of the long bones to ischaemia was most striking in the compactum, especially in the case of the tibia. Many of the vascular canals enlarged until their diameter was several times greater than that of the canals in the controls (Pl. 4, figs. 22, 24). They contained blood vessels with a thin wall composed of a single layer of endothelium and surrounded by a loose reticulum. Active osteoblasts lined the canals in palisade fashion: osteoclasts were rarely seen (Pl. 3, figs. 18, 19). As in the specimens after 1 week of ischaemia, osteocyte death was not present on a large scale: it was found chiefly in the interstitial lamellae of the intermediate zone of the compactum (Pl. 3, figs. 18, 19; Pl. 4, figs. 22–24). Endosteal new bone formation was now well developed, trabeculae being laid down in scattered plaques against the cortex, especially adjacent to ischaemic bone marrow (Pl. 4, fig. 24). Periosteal new bone was also present as irregular deposits. In other areas, the superficial cortical lamellae were deeply pitted by surface erosions containing reticular tissue similar to that found in more deeply situated enlarging canals. Sections cut from ischaemic compact bone where canal enlargement and bone removal were advanced, and stained by Wilder's method for reticulin, failed to show any change in the fibrous organization of the fundamental bone substance when compared with silvered preparations of control material.

Up to 1 month post-operatively the effects of experimental ischaemia on the bone marrow included a drastic diminution in the number of red cells and their precursors in affected areas although eosinophilic granulocytes and plasma cells were very noticeable. Collagen fibres, readily identifiable by their blue coloration in Mallory preparations, and fibroblasts were also conspicuous (Pl. 3, fig. 15, 16), as were groups of multinucleate giant cells (Pl. 3, fig. 17). The latter arose apparently from the coalescence of cells surrounding fat lacunae, possibly the endothelial cells of collapsed sinusoids. Small arteries patent to injected barium sulphate suspension were found in the loose fibrotic infiltrations of the ischaemic medulla (Pl. 3, fig. 15) and were peculiar in that their walls were made up of a single layer of endothelial cells. Control microscopy showed that this is a normal appearance in the rabbit marrow, and that the change over from a thick- to a thin-walled artery is very abrupt.

After the first post-operative month the reactive changes in the marrow rapidly abated, resolution occurring without the formation of scar tissue. The fibroblastic elements vanished together with the multinucleate giant cells, and by 2 months from the start of the experiment the bone marrow appeared normal. On the other hand, from 2 to 5 months enlarged canals in the compact bone remained conspicuous, and came to contain apparently normal haemopoietic tissue in place of the vascular reticular tissue (primary marrow) found earlier. In the tibia especially the relatively huge cortical blood spaces filled with normal-looking haemopoietic marrow (Pl. 4, fig. 25), were striking. Similar changes but lesser in degree were seen in the femur, and warrant the description 'medullization of the cortex'.

DISCUSSION

From this investigation it may be concluded that in the rabbit femur and tibiofibula, experimental ischaemia results in certain major sequelae in both the marrow and cortex. Initially, extensive medullary necrosis occurs with abolition of erythropoiesis in infarcted zones. This is followed by a reactive phase, characterized by the appearance of patent endothelial blood vessels and abundant active cellular elements scattered diffusely through the affected areas. Myelocytes, fibroblasts and multinucleate giant cells are conspicuous in the cellular reaction to marrow ischaemia, but fibrosis did not go beyond the laying down of a loose feltwork of connective tissue fibres. This phase is followed by one of repair wherein abnormal cells and interstitial fibrous tissue disappear, the sinusoidal bed is restored and normal haemopoiesis resumed. On the other hand, the characteristic reaction of compact bone to a diminution in its blood supply is a widening of the cortical vascular canals which come to contain a loose vascular reticulum and osteoblasts. Massive osteocyte death does not occur and the fibrous basis of the fundamental bone substance does not suffer any obvious alteration. Nevertheless, the cortical lesion is progressive, resulting in the removal of large quantities of bone and its replacement by medullary tissue. With medullization of the cortex appears radiographic evidence of osteoporosis and participation of periosteal arteries in the nutrition of the bone as a whole.

The question of the circulatory pathway in bone cortex, ischaemic or otherwise, has been neglected almost universally in the past probably because the terminology employed in describing small blood vessels which pass *into* underlying bone from the periosteal membrane (Pinard, 1952; Morgan, 1959), prejudices the investigator as to the actual direction of blood flow within them. It is interesting, however, to record that Clopton Havers (1691) described the mutrient arteries of compact bone as entering only in localized areas which then give rise to 'vast numbers of veins' leaving the surface of the cortex. Nevertheless, since his day it has generally been taken that normal compact bone possesses in some degree a periosteal arterial blood supply.

Recent investigations into the problems of vascular organization and haemodynamics of long bones (Brookes, 1958 *a*, *b*; McAuley, 1958) lend support to the concept formulated by Brookes & Harrison (1957) of a medullary arterial system responsible for the supply of blood to both the sinusoids of the marrow and the capillaries of compact bone. In normal conditions it is held that the periosteal blood vessels play no part in the arterialization of the underlying bone cortex, but for blood which has flowed through the vascular canals of the compactum a venous drainage pathway is available in the capillaries of the periosteum and the interfascicular capillaries and veins of muscles with a fleshy diaphyseal attachment.

Because the blood vessels of the cortex, and the medullary arterial terminals, are all simple endothelial tubes, it is difficult from histological studies alone to say with any certainty in which direction the blood flows within the cortical vascular canals. A radiological technique was used by Brookes & Harrison not only to visualize aspects of the intra-osseous vascular system, but also to elucidate the problem of blood-flow direction in mammalian compact bone. It was based on the fact that the barium sulphate suspension used did not pass freely into the capillary bed. If the whole length of a capillary from medulla to periosteum is filled, as, for example, in indian ink preparations, the direction of the blood flow cannot be inferred. But by the deliberate employment of an injection mass, such as Micropaque, which only imperfectly enters into the capillary bed, a strong pointer is gained as to the circulatory direction.

In view of the peculiar characteristics of the circulation in bone it is surprising that a curtailment of the normal medullary arterial supply should be in part compensated for by a reversal of blood flow within the compactum, whereby periosteal blood passes centripetally through the cortex into the medulla. The microradiographic results described here, however, show unequivocally that experimental ischaemia, varying in severity, is followed by an appropriate participation of periosteal arterial blood in the nutrition of the affected bone, thus indicating that the periosteal circulation is an important reserve collateral blood route. That blood flow reversal in the cortex is greater in the tibia than the femur in this experiment may be explained as follows: popliteal ligation seriously interferes with the tibial blood supply through the numerous arteries of its upper extremity (Brookes & Harrison, 1957); also when the principal nutrient artery of the femur is eliminated the artery of the trochanteric fossa can take on the role of a second principal nutrient artery (Brookes, 1957) so that ligation of the tibial principal nutrient artery is on anatomical grounds a severer lesion than the corresponding operation in the rabbit femur. The experimental microradiographic findings are also to be correlated with the demonstration in Man (Brookes, 1959) of a significant periosteal arterial supply to ischaemic tubular bone obtained from amputation specimens in cases of peripheral occlusive vascular disease, in which the visual evidence for a pathological centripetal blood flow is in marked contrast to the centrifugal blood flow in normal and quasi-normal human material.

With regard to the histological findings it is noteworthy that few authors have published accounts of principal nutrient artery ligation experiments; Huggins & Wiege (1939), working on the rabbit femur, were the first to record any infarction by this method. Infarction is denied by Kistler (1934) but confirmed by the other authors mentioned. Endosteal bone production is not recorded by Marneffe (1951), but he does describe a mild medullary fibrosis 2 weeks post-operatively, which Bragdon *et al.* (1949) did not observe. The latter deny that haemopoietic function was restored, but Huggins & Wiege were able to report a normal marrow after 74 days of ischaemia without, however, observing the reactive stage of myelofibrosis recorded here as occurring at 2–4 weeks. As for compact bone, all the authors mentioned in this section concur in describing only minimal changes, if any, in the osteocytes of the cortex. Even Kistler in his charcoal embolism experiments would not allow that infarction progressed further than the inner quarter to one-third of the cortex. The present findings show only a slight punctate cellular death in the intermediate zone (where true osteones are found), so it may be concluded that osteocytes in general are particularly resistant to ischaemia. Nevertheless, in order to account for the canal enlargement it seems necessary to postulate some chemical effect on and change within the osteocytes due to curtailment of their blood supply which stimulates the normal canal contents (capillaries and undifferentiated connective tissue elements), into an active phase of bone excavation. This late sequela accounts for the radiographic osteoporosis of ischaemic compact bone found experimentally, and it is suggested that both phenomena are comparable to similar findings reported in ischaemic bones in Man (Jaffe & Pomeranz, 1934; Brookes, 1959). In addition, it would appear that this drastic disturbance to the normal structure of compact bone is an adaptive reaction to adverse environmental change, because the enlarged vascular spaces, now capable of holding *many* blood vessels (and not the usual one for each), permit the periosteal circulation to act as an important collateral blood route. The centripetal blood flow into bone may well be the primary factor tending towards bone survival in ischaemia caused by interruption of the nutrient vessel.

Although not mentioned in this paper, the role of the metaphyseal vessels in affording a collateral circulation for the interrupted nutrient artery must not be forgotten. They may well tide the bone over the fortnight or so before the periosteal centripetal circulation is established.

SUMMARY

1. The principal nutrient artery of the femur and tibiofibula, together with the popliteal artery were ligated and sectioned in adult rabbits, and the sequelae of the ensuing partial ischaemia of bone observed radiologically and histologically over a 5-month period.

2. The results indicate that a reversal of the normal centrifugal arterialization of the cortex takes place to some extent, periosteal arteries participating as a collateral blood route to underlying ischaemic bone.

3. Myelofibrosis and endosteal bone formation are observed as a transient phenomenon up to 1 month post-operatively, the marrow thereafter reverting to normal. The multinucleate giant cells conspicuous in the cellular reaction of the marrow to ischaemia, are possibly derived from sinus endothelium.

4. Osteocytes in partially ischaemic bone cortex do not undergo massive necrosis, but in general retain normal staining properties. The characteristic response of compact bone to ischaemia is a true osteoporosis diagnosable radiologically and histologically, with gross enlargement of Haversian canals which come ultimately to contain haemopoietic marrow, so that a medullization of the cortex results.

I am happy to record my gratitude to Prof. R. G. Harrison for his continued interest in this research and for his many helpful suggestions. My thanks are also due to Messrs A. Taunton and D. L. Reeve for their valuable technical assistance throughout this investigation and in the preparation of the plates.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Radiograph of the undecalcified posterior extremities of a rabbit following 5 months of experimental ischaemia of the right femur and tibiofibula. Barium sulphate suspension has been injected intra-arterially. The bones of the operated right side show greater radiotranslucency than those of the control limb. The vascularization of the right leg is very much denser than normal below the level of the sectioned popliteal artery. ×0·75.

Fig. 2. Microradiograph of a transverse section of a rabbit's tibia 5 months after the production of ischaemia. A pronounced centripetal arterialization of the bone cortex is demonstrated. Many large calibred vessels spring from the periosteal arteries and pass unmistakably into the underlying bone. ×12.

Fig. 3. Microradiograph of a transverse section of the control tibia taken from the same animal shown in Figs. 1 and 2. The stem of the principal nutrient artery is seen in the cortex and its ramifications in the medulla. Fine terminal branches pass centrifugally into the compactum and can be followed at least half-way across its thickness. No periosteal arterial supply is present. ×12.

Fig. 4. Microradiograph of a transverse section through the anterior surface of a rabbit femur rendered ischaemic 4 weeks previously by ligation of the principal nutrient artery. An abnormal centripetal blood flow into the compact bone from the vessels of the attached vastus intermedius is demonstrated. ×12.

Fig. 5. Microradiograph of a transverse section of the control femur to that shown in Fig. 4. The normal radial disposition of medullary arterial terminals and their centrifugal penetration of the endosteal face of the compactum is shown. In spite of dense vascular filling of the attached vastus intermedius muscle no centripetal penetration of the cortex occurs from its periosteal aspect. ×12.

PLATE 2

Fig. 6. Microradiograph of a longitudinal section through the diaphysis of a rabbit tibia 3 months after the production of bone ischaemia. Numerous large calibred anastomoses between periosteal and medullary circulations are shown in which the blood flows centripetally through the cortex into the medulla. ×13.

Fig. 7. Microradiograph of a longitudinal section through the control tibial diaphysis to the one shown in Fig. 6. Medullary arteries are shown coursing in the outer zone of the bone marrow giving off terminal branches which pierce the cortex centrifugally and are traceable about half way across its thickness. Periosteal structures are still *in situ*. Along the lower edge of the print the central venous sinus can be seen. ×13.

Fig. 8. Microradiograph of diaphyseal marrow taken from a femur three weeks after ligation of the principal nutrient artery, showing medullary sinusoids arranged in a lobular fashion. × 15.

Fig. 9. Microradiograph of the vascular contents of diaphyseal marrow seen in an ischaemic rabbit femur 3 weeks after operation. The medullary sinusoids form a dense reticular background against which discrete arterial channels are seen. × 15.

Fig. 10. Microradiograph of a transverse section of an ischaemic rabbit tibia 2 weeks after operation. An abnormal centripetal periosteal arterial supply to the cortex has been revealed by intra-arterial injection of radiopaque suspension. Much of the marrow still retains a blood supply, although in this section a large infarcted area is shown, demarcated by injected medullary sinusoids. × 10.

Fig. 11. Microradiograph of a transverse section through the ischaemic tibia of a rabbit 2 months after operation. A large calibred arterial channel passes centripetally through the cortex opening directly into medullary sinusoids. × 15.

Plate 3

Fig. 12. Photomicrograph of normal rabbit femoral marrow showing a portion of the central venous sinus, an adjacent large medullary artery and haemopoietic blood spaces. Mallory, × 110.

Fig. 13. Photomicrograph of the femoral medulla of a rabbit 1 week after section of the principal nutrient artery. Many of the marrow sinusoids are collapsed; the artery shown in cross-section is empty of blood and radiopaque medium, but some haemopoiesis is still occurring. Mallory, × 110.

Fig. 14. Photomicrograph of ischaemic marrow in a rabbit tibia 2 weeks after operation. Many fat lacunae have vanished and haemopoietic areas replaced by fibroblastic infiltrations. Several multinucleate giant cells are also visible in this preparation. Haematoxylin and eosin, × 110.

Fig. 15. Photomicrograph of the bone marrow taken from the midshaft of a tibia 2 weeks following production of ischaemia. Two small medullary arteries filled with Micropaque are shown whose wall is a single layer of endothelium. Fibroblasts, plasma cells and eosinophilic myelocytes are found in abundance in the myelofibrotic infiltrations. Haematoxylin and eosin, × 430.

Fig. 16. Photomicrograph of tibial bone marrow after 4 weeks of ischaemia. Islands of medullary bone are shown developing in fibrotic marrow. Mallory, × 130.

Fig. 17. Photomicrograph of a cluster of multinucleate giant cells developing in the tibial bone marrow of a rabbit 2 weeks after production of ischaemia. Several stages are shown in the formation of these giant cells, which appear to be derived from sinus endothelium surrounding the fat lacunae which are undergoing replacement. Haematoxylin and eosin, × 250.

Fig. 18. Photomicrograph of an enlarging Haversian canal in an ischaemic rabbit tibia 4 weeks after operation. The canal contains small endothelial vascular tubes in a loose reticulum bounded by a pallisade of osteoblasts. Osteocytes in the vicinity of the canal appear normal. Haematoxylin and eosin, × 480.

Fig. 19. Photomicrograph of two osteoclasts in the ischaemic tibial cortex of a rabbit, 3 weeks after operation. Although the bone is undergoing active dissolution, the osteocytes appear normal. Haematoxylin and eosin, × 480.

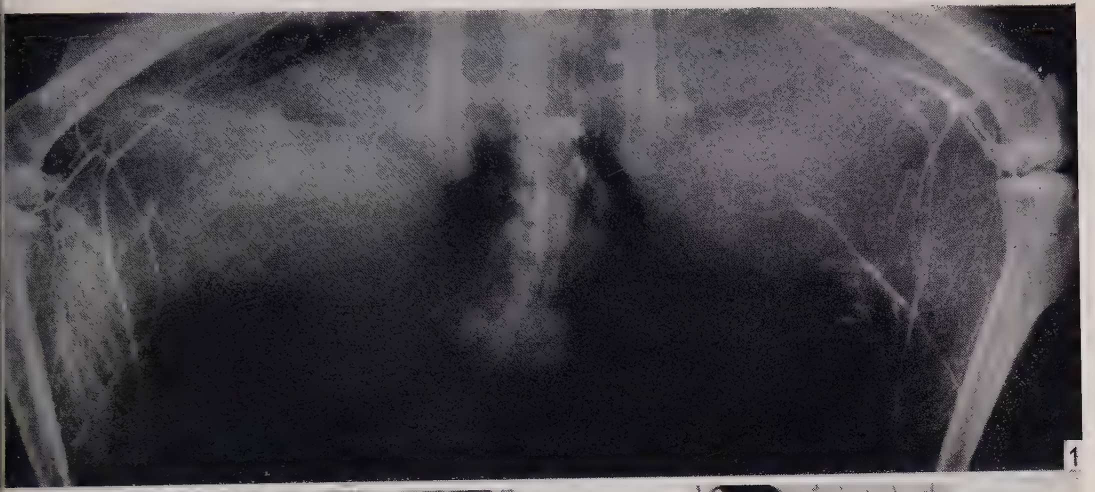
Plate 4

Fig. 20. Photomicrograph of a transverse section of the control tibial cortex of a rabbit in which the contralateral tibia had been rendered ischaemic 1 week previously, showing normal Haversian canals and osteocytes. Mallory, × 110.

Fig. 21. Photomicrograph of a transverse section of ischaemic tibial cortex in a rabbit 1 week after operation showing enlargement of some Haversian canals in a field of normally staining osteocytes. Mallory, × 90.

Fig. 22. Photomicrograph of rabbit tibial cortex after 2 weeks of ischaemia, showing considerable enlargement of the Haversian canals. The osteocytes appear normal in general, poor staining tending to occur in interstitial lamellae. Mallory, × 90.

Fig. 23. Photomicrograph of ischaemic tibial cortex 2 weeks after operation showing enlarging Haversian canals and normally staining osteocytes. Haematoxylin and eosin, × 470.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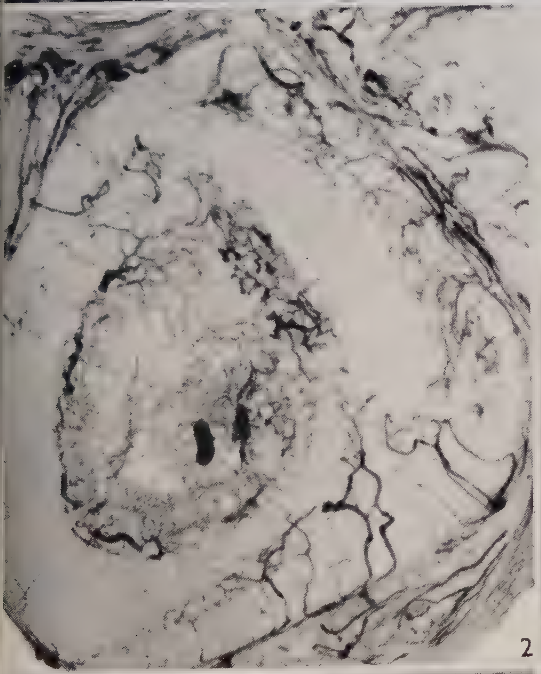
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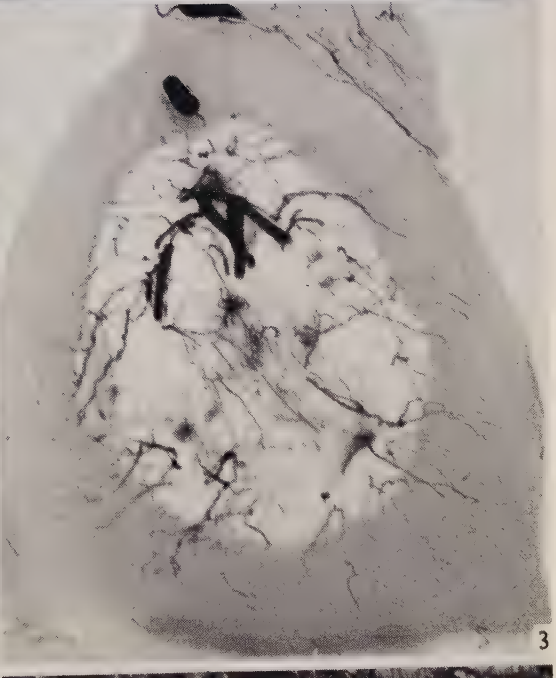
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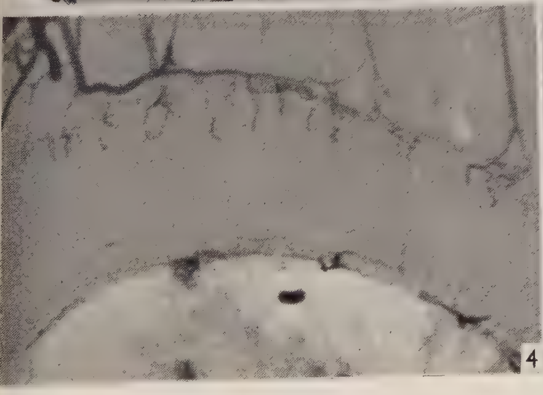
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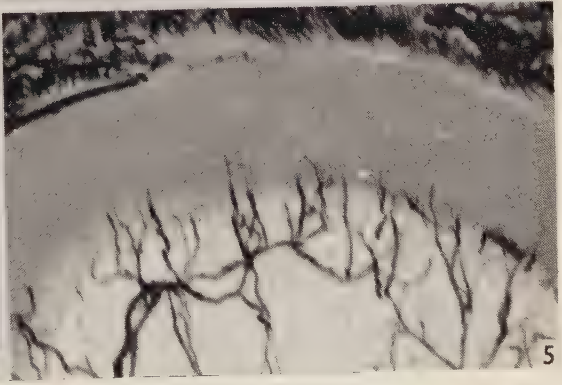
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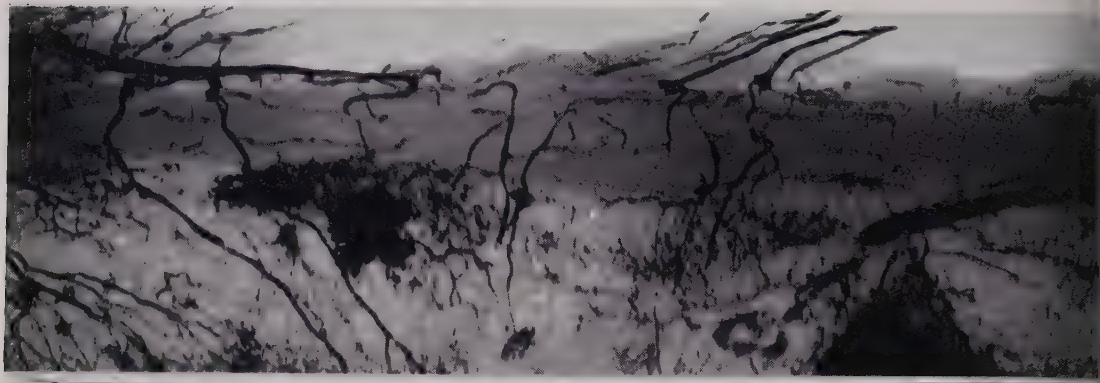
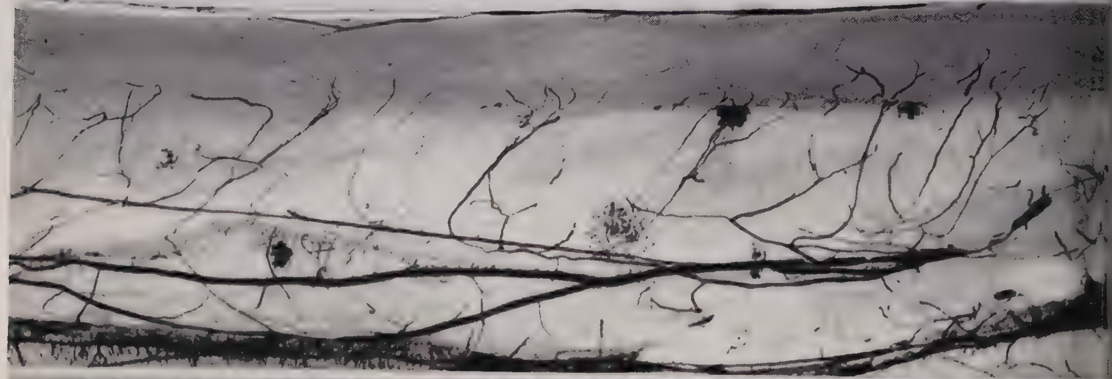
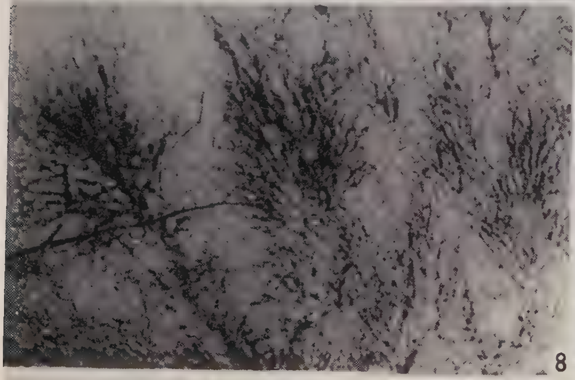
BROOKES—SEQUELAE OF EXPERIMENTAL PARTIAL ISCHAEMIA IN THE RABBIT







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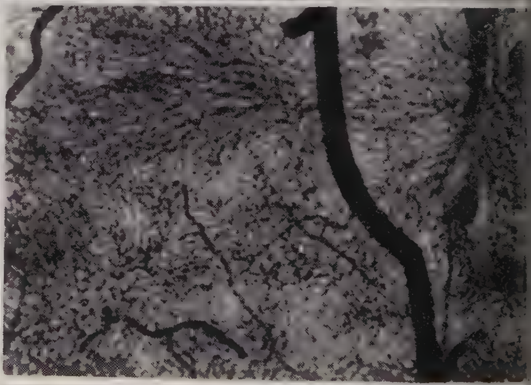

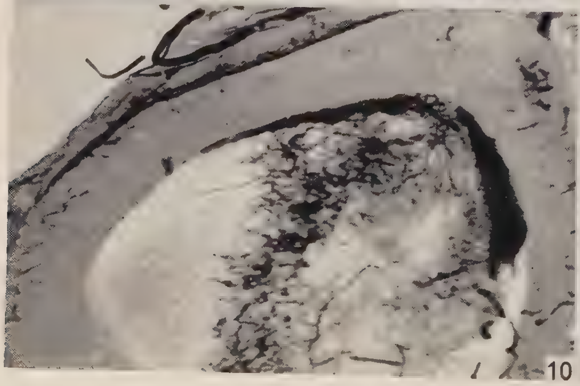

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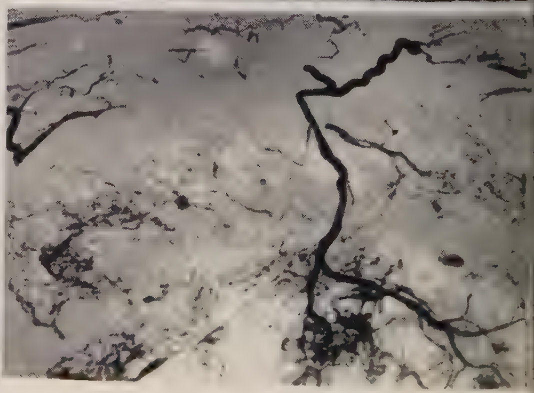
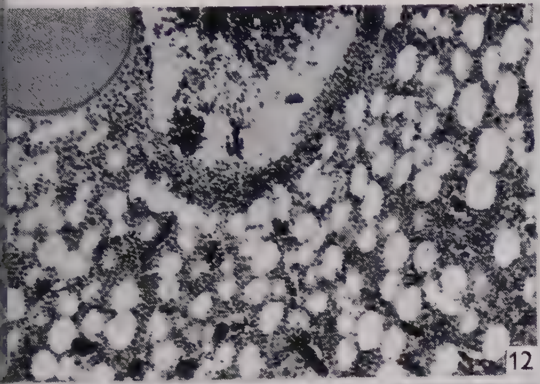


BROOKES—SEQUELAE OF EXPERIMENTAL PARTIAL ISCHAEMIA IN THE RABBIT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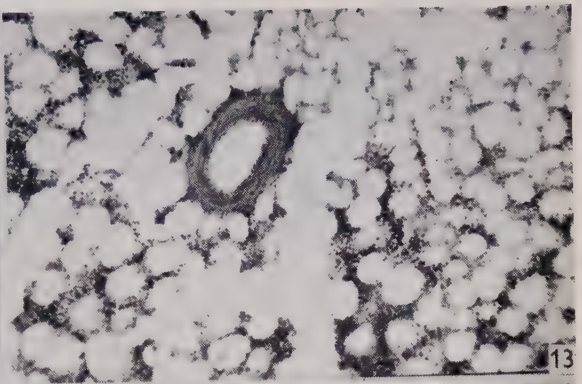
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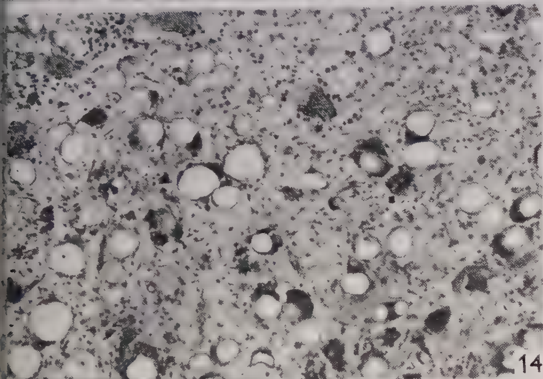
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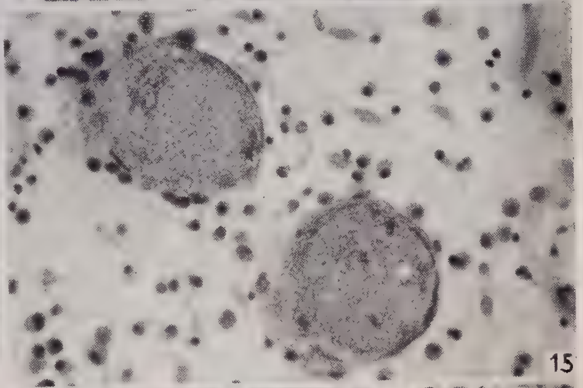
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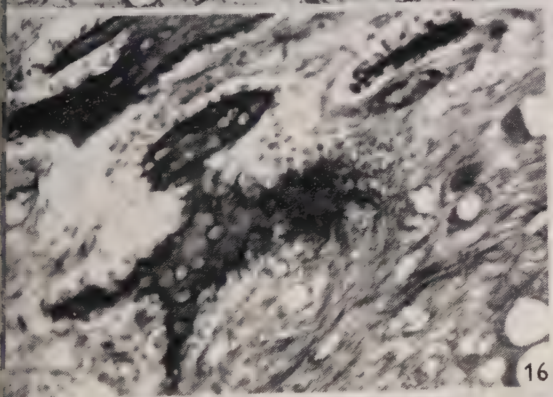
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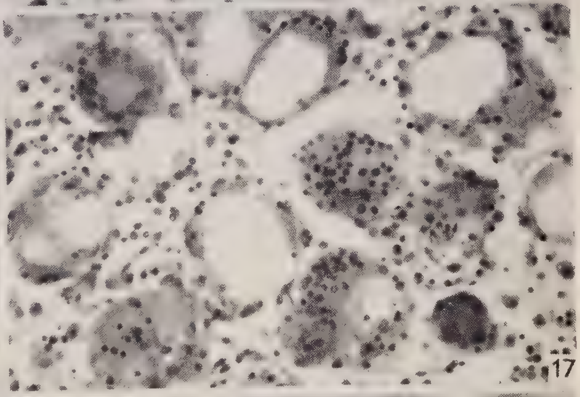
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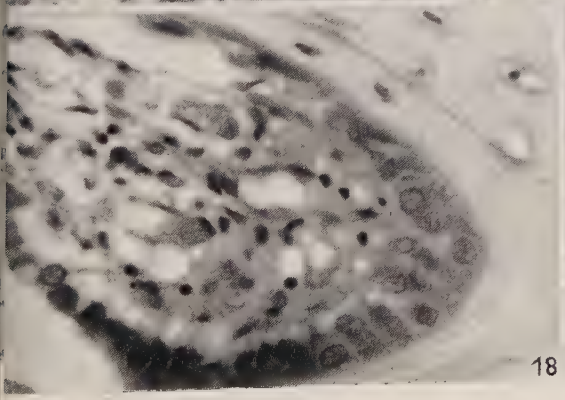
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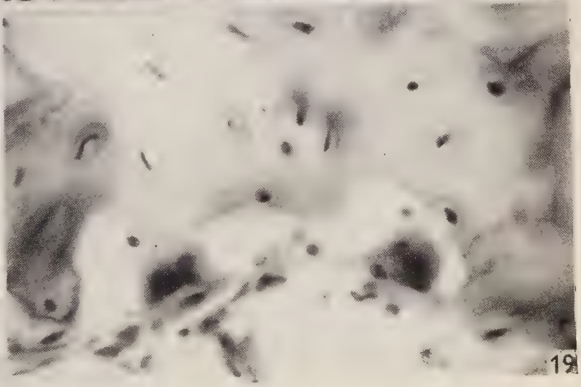
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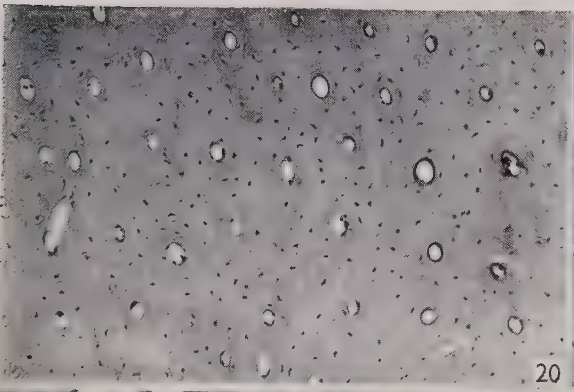
BROOKES—Sequelae of experimental partial ischaemia in the rabbit



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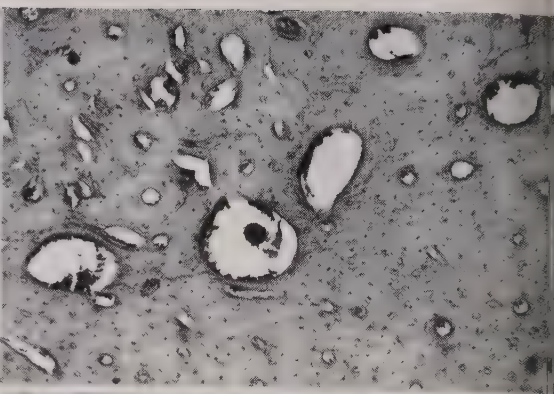

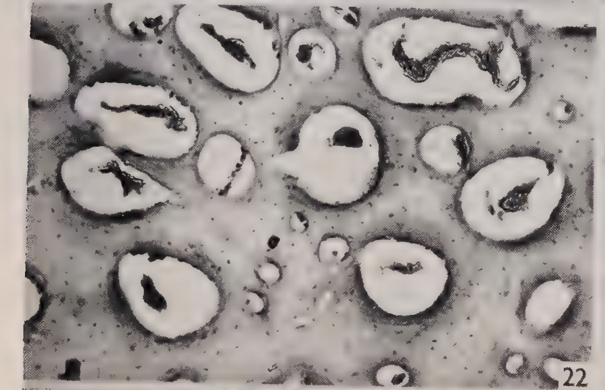

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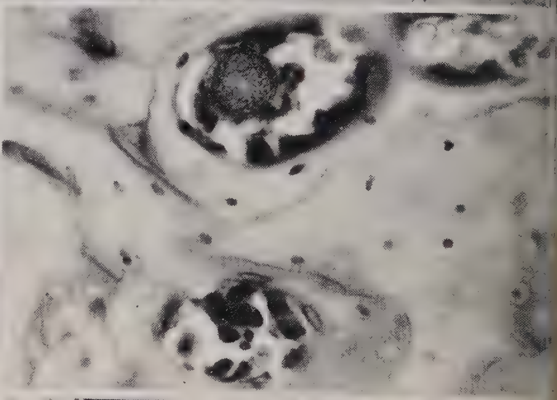

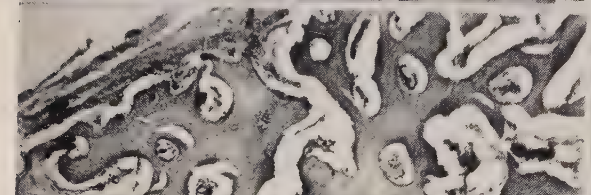

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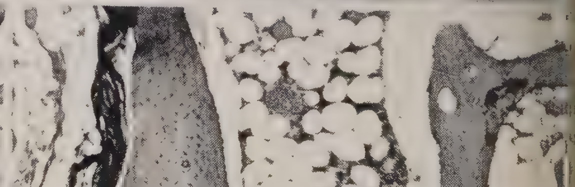

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BROOKES.—SEQUELAE OF EXPERIMENTAL PARTIAL ISCHAEMIA IN THE RABBIT

Fig. 24. Photomicrograph of a transverse section through the ischaemic tibia of a rabbit 2 weeks after operation, showing a generalized enlargement of the Haversian canals which contain a loose vascular tissue and osteoblasts, frequently in pallisade formation. A plaque of newly formed endosteal bone is seen towards the lower left-hand corner of the illustration where ischaemic marrow is also visible. Massive osteocyte death has not occurred. Haematoxylin and eosin, ×40.

Fig. 25. Photomicrograph of a transverse section through the tibial cortex of a rabbit rendered ischaemic 5 months previously. Giant spaces are present in the otherwise compact bone, and contain apparently normal haemopoietic marrow. The osteocytes in the field are in general normal in appearance. Haematoxylin and eosin, ×60.

IN MEMORIAM

C. C. MACKLIN

On 23 November 1959, Charles Clifford Macklin, Professor Emeritus of Histology and Embryology, University of Western Ontario, died suddenly and without warning at London, Ontario, in his 76th year.

His death recalls an era of experimental histology of which he was a leader bringing honours not only to himself, but also to the medical school at London, Ontario, where for 32 years he held the Chair in Histology and Embryology. He contributed greatly to the science of microscopic anatomy during a period when all too frequently the discipline tended too much to pure description *in vacuo*. But the researches of Prof. Macklin, along with those of Bensley, Hoerr, Terry and Wislocki, never failed to delineate their morphologic discoveries in meaningful relation to physiology and endocrinology. Had it not been for Charles Macklin and his peers, the high standard of microscopic anatomy achieved in this country might never have been maintained.

Born on 23 October 1883 in the village of Tod Morden, now a part of the city of Toronto, Ontario, he came of pioneer Scottish stock which had first settled in Ulster because of the persecutions of the Scottish Kirk. After receiving his primary education, he at first worked in the Medical Department of the Canada Life Assurance Company before deciding to enter the study of medicine. Not having a high school education he engaged a tutor who taught him three nights a week and he prepared his work the other three. Even though he was working full-time in the office during the same period, within nine months he completed his entire junior matriculation and entered the School of Medicine at the University of Toronto in 1908. Ranking among the first four of his class, he won the James Richardson Fellowship in Anatomy which proved to be the turning point in his career and it enabled him to complete an excellent paper on the embryology of the human skull (*Amer. J. Anat.* **16**, 317, 1914). His high scholastic standing and this excellent work won him an invitation to join the Anatomical Staff at Johns Hopkins Medical School. With the outbreak of the war he entered the services of the United States Army doing contract surgeon's work until 1918 on the treatment of head wounds under Captain Lewis Hill Weed on the staff of Anatomy at Hopkins.

At that time the degree of M.D. (Toronto) went to only those completing outstanding research and in 1916 Dr Macklin was awarded this honour.

He married Madge Thurlow, a final year student in medicine at Hopkins, in the fall of 1918. She worked side by side with him for many years. An outstanding scientist in her own right in the area of human genetics she survives the death of her husband and to her and her three daughters we extend every sympathy.

After a brief period on the staff of Anatomy with rank of Professor at the University of Pittsburgh, Dr Macklin returned to Johns Hopkins in charge of Gross Anatomy with his wife an Instructor in the Department of Physiology in the School of Hygiene. His stay here, however, was only until 1921 for he felt strongly

C. C. MACKLIN

(Facing p. 562)

that Canadian scholars should not go to the United States, but make their contributions to the best of their ability in the land of their birth. So in 1921 he returned to Canada as Professor of Histology and Embryology at the Medical School of the University of Western Ontario in London, Ontario, and there he remained until retirement. Within three years he was elected to the Royal Society of Canada, an outstanding honour for one so young. He belonged to the American Association of Anatomists, on whose Executive Board he served and he was later made a Life Member of the Anatomical Society of Great Britain and Ireland upon his retirement in 1953. In addition, he was an active and contributing member to the Anatomische Gesellschaft of Germany, to the Canadian Medical Association, to the Canadian Physiological Society, to the Marine Biological Laboratory, which also made him a Life Member on his retirement and to the American Association for the Advancement of Science.

In addition, he gave outstanding presentations before several International Congresses. Twice (1931 and 1942) he gave the James Gibson Memorial Lecture in Anatomy at the University of Buffalo and, in 1950, the 26th Lewis Linn McArthur Lecture of the Frank Billings Foundation of the Institute of Medicine in Chicago. His chapters in the volumes of Cowdry's *Special Cytology* (1927) and *Aspects of Aging* (1944) are landmarks in their subjects.

A grateful Medical School gave him the honorary degree of Doctor of Science of the University of Western Ontario on his retirement in 1953, and in 1959 only a few months before his death, he was invited to attend a Symposium on Emphysema in Aspen, Colorado, which would have been held in his honour had he consented to accept. But ill health prevented him from doing so.

As one who had the privilege of working under Prof. Macklin for several years in the early part of the Second World War, it is possible to speak of his never flagging intellectual curiosity and critique of every phenomenon encountered in the laboratory. Probably his researches on the etiology and pathogenesis of pulmonic and mediastinal emphysema illustrate his unique talents most vividly. The techniques he devised were simple and direct and his instruments a far cry from the elaborate machines that crowd our contemporary laboratories. Yet with little more than his hands, his eyes and his microscope he made contributions in the field of pulmonic histology that immeasurably advanced our understanding of the structure and the function of the lung. Furthermore, he imparted much of this philosophy to the younger men working with him and for this they will be always in his debt.

Prof. Macklin's research was not only his work but also his hobby and indeed his life. Reading poetry, Shakespeare's plays and listening to great music provided the relaxation necessary to such an active intellect. We mourn the passing of one of the great histologists of our time and one of Canada's leading scientists.

W. S. HARTROFT

IN MEMORIAM

D. SHOLL, B.Sc.

By the death of Donald Sholl we have lost one who has made a most distinctive contribution to Anatomy in recent years. He came to the subject late in life, after a training at University College in Statistics. He was not a brilliant mathematician, and indeed apparently an unsatisfactory student. For years he moved from one subject to another; from theology to physiology, then to zoology and to statistics, without much impressing anyone. But as it later proved he was all the time searching in his own way for the work that he should do. He came back to University College in 1947 to work with Professor de Beer on problems of growth of the brain. He was soon able to show that most of the conclusions about the relative size of brain and body, confidently asserted by du Bois and others, are not valid deductions from the scanty evidence of the small numbers available. Unfortunately these unfounded laws are still widely quoted, perhaps because it was not in Sholl's nature to make loud and long propaganda about negative conclusions.

This work, however, brought him into contact with neurology and he began to realize that in the study of the brain he could find the focus that he had been seeking for his activity. His next work was undertaken in connexion with studies on the growth of nerves and was a truly remarkable investigation of the relative rates of growth of mandible, arm and leg (*J. Anat., Lond.*, **83**, 296, 1949, with R. H. Shepherd & R. Vizoso). Again he was unable to fit the results to an orthodox interpretation; since the length of each of these parts remains a linear function of sitting height from birth to maturity, there is no 'allometry'.

At this time he also collaborated with Professor Ruth Bowden in her study of the rate of advance of recovery after peripheral nerve injury. These data present serious problems and he showed how much can be done when methods that may be quite ordinary to the statistician are applied by someone with the patience to think out what features the investigation really requires to establish.

It was the brain, however, that really interested Sholl, and he began at this time the work on cortical dendritic patterns that was his greatest contribution. The problems of the differences between cortical areas clearly demand a quantitative approach but, as other investigators have found, the problem is 'what to measure?'. The impact of Sholl's attack on the problem was that he realized that it is only possible to think of cortical organization itself in statistical terms. The problem is thus not only what is the statistical validity of alleged differences between areas, but the much wider one of how to express the functionally relevant features of this organization. His thoughts along these lines provided a stimulus to a wider range of people than it is given to most anatomists to reach. His book on *The Organization of the Cerebral Cortex* has a character quite its own. Its method of treatment has been adopted by physiologists and engineers all over the world, as well as serving to show for the first time how the results of the beautiful Golgi technique may be made quantitative.

Such contributions, all within little more than ten years, show the remarkable concentration of effort that lay behind his quiet, almost lazy manner. The secret was that he was not afraid to select the work and methods that he would use, with no regard for what was fashionable. His belief in putting first things first may have made him sometimes unconventional, but it provided, for many of us, a constant example of how judgement should be used. This was true especially for his pupils. He played a particularly important part in the expansion of teaching for the B.Sc. Special Degree in Anatomy at University College, and, indeed, in London University in general. He knew how to help the medical student to escape from the suffocation of work for the 2nd M.B., by setting him to study books and specimens for himself. There are many who owe it largely to Sholl that they were given the opportunity for expansion of their outlook and abilities, that a university education should provide. No effort was too great in helping with work in College or entertaining at home. Besides his own contributions to Anatomy, he has left a generation of young medical scientists who will carry on his work as he would have wished.

J. Z. YOUNG

REVIEWS

Peripheral Facial Palsy: Pathology and Surgery. By Karsten Kettel. (Pp. 341; 127 figures; £6.) Oxford: Blackwell Scientific Publications, 1959. (Original Publisher Ejnar Munksgaard, Copenhagen, Denmark.)

In the author's opinion the loss of 'the language of facial expression' is so grave a handicap that every effort must be made to preserve or restore the functional continuity of the seventh nerve. This monograph presents the fruits of his meticulous study of 311 cases of peripheral facial palsy in which surgical intervention had been undertaken. The nerve was decompressed in 233 instances, sutured in 5 and repaired by a graft in 70.

The work is in three parts, the first deals with general considerations of the anatomy and physiology of the nerve, the classification, diagnosis, localization and conservative and operative treatment of the lesions. Atraumatic and traumatic palsies are considered in detail in the second and third parts respectively. The clinical picture, pathology and treatment of many conditions are described with reference to the findings of others and each chapter is summarized and conclusions are drawn, a practice which is useful when the book is used as a *vade mecum*, but one which becomes repetitive when the book is read as a whole. Seddon's classification of paralytic lesions is discussed, although the text shows that the author makes no distinction between uncomplicated axonotmesis and lesions in continuity with some disturbance of the internal architecture of the nerve trunk.

Electromyography has been available for only a small number of cases and strength/duration curves do not appear to have been used, therefore most of the discussion on electrodiagnosis is based on less reliable faradic and galvanic stimulation of the muscles. No mention is made of diagnostic percutaneous stimulation of the nerve trunk below the level of injury, a procedure which gives the earliest evidence of a degenerative lesion since conduction fails within about 3 days after severance of the axons; whilst persistence of nerve conduction in the presence of paralysis is indicative of neurapraxia. Although electrodiagnostic findings form only a part of the clinical picture, serial quantitative tests, according to Collier, may obviate operation is selected cases of Bell's palsy without detriment to the patients. However, on the basis of his own experience the author is reluctant to accept this view and like Cawthorne, who writes the foreword, favours operative rather than expectant treatment. An ischaemic aetiology of Bell's palsy is discussed at length and autonomic imbalance due to disturbance of the parasympathetic system is postulated as a causal factor, but the evidence for incriminating the parasympathetic, rather than the sympathetic system is not divulged.

The book gives an authoritative account of the anatomical variations in the course of the nerve; cases are reported fully and are lavishly illustrated by photographs of the pre- and post-operative conditions. Operative technique is discussed and coloured photographs of operative findings are included; the value of the latter would have been enhanced by better labelling. Finally, the results of treatment are surveyed critically and the author analyses the causes of his few failures in this exacting but rewarding field of surgery. It is unfortunate that so important and costly a book should be marred by numerous misprints.

R. E. M. BOWDEN

Encyclopaedia of Microscopic Stains. By EDWARD GURR, F.R.I.C., F.L.S., M.I.BIOL., F.R.M.S. (Pp. 498 + xii; 95*s.*) London: Leonard Hill (Books), Ltd. 1960.

Methods of Analytical Histology and Histo-Chemistry. By EDWARD GURR, F.R.I.C., F.L.S., M.I.BIOL, F.R.M.S. (Pp. 327 + xv; 7 illustrations; 70*s.*) London: Leonard Hill (Books), Ltd. 1958.

The foreword to the encyclopaedia compares the progress from materia medica to scientific pharmacology with the change from empirical staining methods to histochemistry. This book, which presents the structural formulae, molecular weights and various solubilities of all the usual staining reagents, should accelerate and facilitate this change. The stains are listed alphabetically, and the author uses his great experience to add informative and well documented footnotes.

The book on methods describes, accurately and simply, the technical procedures for all the common histological and histochemical reactions. The unusually clear presentation and the inclusion of important trivial details makes this book ideal for a technician to follow on his bench. Each group of biological substances is introduced by a biochemical summary and the individual techniques are followed by attempts to explain their chemical basis. Minor variations in techniques, such as the extraction methods for nucleic acids and the reactions for adrenaline and noradrenaline, are described as different methods with unnecessary repetition, and where more than one technique is given there is usually no attempt to indicate preference. Although this book will be useful, it is the encyclopaedia which makes a unique contribution to histology.

A. R. MUIR

Ciba Foundation Colloquia on Ageing. Volume 5.

The Lifespan of Animals (Pp. xii + 324; with 58 illustrations and cumulative index to volumes 1–5; 48*s.*) J. and A. Churchill Ltd. 1959.

The papers in this fifth and last volume of the present series of Ciba colloquia on ageing deal with the lifespans of mammals, birds, fish and insects. The emphasis in several of the articles is distinctly actuarial, and although the participants are agreed that the term 'lifespan' cannot be concisely defined there is much interesting, if heterogeneous, information on the length of life in a wide range of creatures. There is disappointingly (though not surprisingly) little information as to why lifespan is so variable between species, although the articles by G. A. Sacher, who makes an ingenious attempt to relate longevity to brain and body weight and by H. S. Simms, B. N. Berg and D. F. Davies, who correlate longevity in rat and man with the onset of disease, provide useful ideas for further research. A. Comfort's study of the longevity of English thoroughbred horses (compiled from the *General Stud Book*) gives perhaps the fullest information yet on the lifespan of a large mammal other than man. R. J. H. Beverton and S. J. Holt give an excellent review of the lifespan of a large range of fish in nature, and this is supplemented by R. F. Nigrelli's account of the longevity of fish in captivity; while S. D. Gerking describes changes in the metabolic and reproductive functions of fishes with age.

The three papers on ageing in insects by A. Maurizio (bees), M. Rockstein (bees and houseflies) and J. Maynard Smith (Drosophila) fall in a different category from the rest because of the great differences between insects and vertebrates in development and physiology, but provide a stimulating contrast to the vertebrate studies.

The other communications deal with actuarial aspects of human lifespans (B. Benjamin), parental age effects in man (A. Jalavisto), lifespan of cattle and horses (W. Hartwig), lifespans of mammalian and bird populations in nature (F. Bourlière) and arteriosclerosis in birds (S. Scheidegger). For the mathematically minded there is an appendix by R. E. Beard on some mathematical mortality models.

This colloquium illustrates both the difficulty of determining the lifespan of animals in a natural state, and the enormous gaps in our knowledge of the basic biological causes of ageing, and because of this the mathematical speculations tend to surpass the solid basis of biological facts on which they depend. Nevertheless, the large quantity of hard-won data and ideas which this volume contains will make it indispensable to anyone interested in ageing.

E. H. R. FORD

Genetic Basis of Morphological Variation. By R. H. OSBORNE and D. V. DE GEORGE. 48*s.* London: Oxford University Press. 1960.

Present knowledge of human genetics relates mainly to certain major factors which can be easily identified by their clear-cut phenotypic effects. Little is known of the genetic background of continuously-varying human characters, although the observed statistical correlations on the one hand between parents and offspring, and on the other between sibs, support the view that in most cases phenotypic variation is due in part to environmental influences and in part to the cumulative effects of numbers of minor genetic factors. Studies of these minor factors are beset with technical difficulties, but such information as can be obtained is significant because of its relevance to an appreciation of the genetic background of the bony features on which an assessment of the taxonomic status of human and primate fossils rests.

Comparison of the variation between like and unlike twin pairs provides an approach to the study of the genetic basis of continuously-varying human characters, for if the variance within dizygotic twin pairs (i.e. pairs whose members are genetically dissimilar and in which variation may result from both genetic and environmental influences) is significantly bigger than that within monozygotic twin pairs (i.e. pairs which are genetically identical, and within which phenotypic variation results only from the action of environmental factors), there is presumptive evidence that genetic factors are to an appreciable extent operative.

Further evidence for the genetic control of any feature is provided first if the variation between dizygotic twins of unlike sex is bigger than between pairs of the same sex and second if the variation within dizygotic twin pairs is less than that between such pairs.

As an extension of this concept, it is submitted that if the statistical correlations between two characters are significantly bigger within single individuals than between like twins and if these are in turn bigger than the corresponding correlations between unlike twins, it is probable that both characters are influenced by the same constellation of genetic factors.

The present study, which is based on an examination of 131 pairs of adult twins—61 of the dizygotic variety and 70 of monozygotic origin, contains analyses of a number of standard anthropometric measurements including some which relate specifically to the development of muscle, bone and fat. In an attempt to examine the genetic background of overall bodily proportions the subjects are graded according to the somatotype scale, while in order to assess the overall genetic control of sexual differences in bodily dimensions, a 'masculinity and femininity rating' is computed and compared, first in dizygotic twins of the same sex and second in twins of unlike sex.

The main conclusion to which the study points is that different measurements vary markedly in the extent of their genetic control, measurements of girth together with those taken parallel to the long axis of the body apparently being the most strongly influenced. Correspondingly, the development of fat, bone and muscle is affected to an appreciable degree by genetic factors.

The results, although significant, are less clear-cut when groups of characters are considered simultaneously. There is evidence first that in the case of the somatotype rating the three components have some genetic validity but 'their particular combinations can not be shown to have a genetic basis'. Second, the masculinity–feminity rating 'makes it possible. . .to demonstrate statistically a genetic component of variability for morphological bisexuality'. Third, the genetic interrelationships between pairs of linear measurements are, as shown by comparisons of statistical correlations, highly complex.

The study is presented in a concise, scholarly style and the care and criticism that have characterized each stage of the analysis make an immediate impact. The reader can, for instance, scarcely fail to be impressed by the authors' appraisal of the constitution of the available twin samples, by the rigorous criteria used for separating monozygotic and dizygotic twin pairs and by the precision of the anthropometric techniques. The statistical analyses are simple and the cautious interpretation of the results endorses the view expressed in a foreword by Prof. Dobzhansky that the present monograph represents 'a great step forward in the studies on the nature-nurture problem'. Nevertheless, as the authors rightly stress, much information remains to be extracted from analyses of this type. Additional comparable data would in some instances be useful to reduce sampling errors, but further advances in the study of the genetic control of size and shape would appear to depend to a considerable extent on the application of techniques of multivariate analysis. Although firmly established in theory, the use of these methods is still limited by the elaborate computations that are involved.

E. H. ASHTON

The Microcirculation. Factors Influencing Exchange of Substances across Capillary Wall. Edited by S. R. M. Reynolds and B. W. Zweifach. (Pp. v+170. $4.50.) Urbana: University of Illinois Press 1959.

This volume consists of the papers delivered at the fifth Microcirculatory Conference held in 1958. They include papers on general topics such as the morphology of endothelium as seen by electronmicroscopy, the dynamic forces involved in capillary permeability and the properties of artificial membranes and pores as well as articles on more specific subjects such as the relationship between size and distribution of tumour metastases, the circulation in the small intestines, lungs and skin and the permeability of lymphatics to albumen. The problems still to be solved become all too evident as one reads the volume as does the gap which exists between the morphological and dynamic concepts. These should provide a stimulus and a challenge to beginners, both in the anatomical and physiological fields. The volume should, however, have a much wider appeal. The articles are all by experts and provide an up-to-date summary of the advances of our knowledge in important aspects of a topic which directly or indirectly impinges on most anatomical, physiological and pathological research and teaching.

D. V. DAVIES

A Textbook of Human Embryology. By R. G. Harrison. (Pp. xi+244; 144 illustrations; 45*s*.) Oxford: Blackwell Scientific Publications. 1959.

This book, based on courses of lectures given by the author, is designed primarily for the medical student. The bold outlines of the developmental story are given; and, with a view to making the subject stimulating, easily assimilable, obviously useful but still comprehensive, much morphological detail has been omitted or condensed. The student is encouraged to treat 'development' as something which can be grasped even by a student; and in this he is aided by the use of the large type, good lay-out, plentiful line diagrams, and photomicrographs of human embryos. An introductory chapter is followed by excellent chapters on the male reproductive system and spermatogenesis. The author has drawn freely on his own researches to illuminate these and other chapters. Succeeding chapters deal with the female genital system, the roles of the pituitary and adrenal glands, early embryonic development, the placenta, the development of the body systems and organs, and postnatal regeneration of damaged tissues. Useful and very recent references are given at the end of each chapter; author and subject indices complete the book. Some confusion in the minds of students may result from the use of involved sentences and a somewhat colloquial style. For example, the account of the development of the pars phallica of the male urethra (p. 168) is most confusing to read. The use of the words 'obvious' and 'therefore' should be restricted to statements that are obvious or the logical outcome of events just described. It is neither obvious, nor true, to say (p. 85) that: 'The most obvious structure to develop from ectoderm is, of course, the skin.' The author does immediately afterwards go on to deal with the two fundamental layers from which the skin is developed, but the damage is done. The most notable omission from the book is a consideration of the conducting system of the heart. The only misprint observed is that Saxén's name is misspelt throughout; and one reference (Legait, 1957, p. 194) is omitted from the list at the end of chapter XXI, and from the author index. The general standard of presentation is good, but it would be an improvement if all the illustrations of transverse sections of embryos were uniformly oriented. Not all the assertions made in this book will be completely acceptable to other anatomists, but in spite of this the book should prove useful as an introduction to human embryology and as a guide to further reading.

W. R. M. MORTON

Pratique Anthropologique. By GEORGES OLIVIER. (Pp. 292; 73 figures.) Paris: Vigot Frères. 1960.

This is a useful hand-book of practical anthropology containing an adequate and clear description of most of the basic measurements commonly used in the anthropometry of either the living or the skeleton, together with the most frequently used indices to be derived from them. The text contains the names and definitions of the points from which the measurements are made and the accompanying line drawings facilitate their identification, but no descriptions of the technique of measurement are given.

The book contains much useful information, such as a table of cranial indices, sexual differences, ossification dates of limb bones, as well as a section on elementary statistical technique. There is a useful bibliography at the end of each section as well as a general one at the end of the volume. The quality of the paper and reproduction are good.

E. W. T. MORRIS

Schémas de Travaux Pratiques Ostéologie et Arthrologie. By GEORGES OLIVIER. (Fascicule I, *Le Squelette Appendiculaire*: 114 pages; 1250 Frs. Fascicule II, *Le Squelette axial*: 84 pages; 950 Frs.) Paris: Vigot Frères. 1959.

This work, designed to assist the study of osteology and arthrology, is in two parts; Part I dealing with the appendicular and Part II the axial skeleton. It consists of labelled drawings of bones and joints. The drawings are clear, accurate and well reproduced, and each bone is drawn from several aspects so that its important features are seen and the student should be able to identify them on the actual bone. Accompanying each long bone are simple instructions for placing it correctly in the body. The drawings include longitudinal sections to display the trabecular pattern of the spongy bone in relation to normal stresses.

The illustrations of joints clearly show the disposition of ligaments and there are additional ones showing the relation of arteries and nerves as well as some line drawings of radiographs taken from different aspects of the joint.

The author suggests that the student should add to the drawings of each bone the muscles, nerves, vessels and ligaments attached or related to it. They should then form a valuable and compact source for reference or revision to any student who has learned his osteology with the aid of his half set of bones, the osteological section of a standard text-book of anatomy and these drawings.

The French nomenclature may cause some difficulty; students may not recognize teres major in grand rond. There are several errors of labelling such as on p. 22, Part I, where the long heads of biceps and triceps are transposed. There are a few factual errors such as the relation of the infra glenoid tubercle and the long head of biceps to the capsule on p. 33, Part I, and not all anatomists would agree with the schema of scapular movements on p. 26 and the abduction of the arm on p. 37, Part I.

E. W. T. MORRIS

Grundlagen der Anatomie. By Kurt Alverdes. (Pp. xx+841; 195 illustrations; gebunden DM. 57). Leipzig: VEB Georg Thieme. 1959.

The aim of the author of this book is to provide the student with a concise text-book of Anatomy which would also serve as a bridge from preclinical to clinical studies. The book is well illustrated and the majority of figures are in colour. The description of muscles, blood vessels and the peripheral nerves is given in tabular form. The chapter dealing with the central nervous system and its pathways is well arranged and clearly presented. No references are found to embryology, but useful comments are added throughout the text on the clinical application of the anatomical facts. The book is evidently appreciated by students since it has reached its second edition in three years. N. CAUNA

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

JULY 1960

The Summer Meeting of the Society for the Session 1959–60 was held on Friday, 1 July 1960 in the Anatomy Department, the Royal College of Surgeons, Lincolns Inn Fields, London, W.C. 2.

The President (Prof. J. D. Boyd) and Prof. F. Goldby and Prof. G. Causey occupied the Chair at the various Sessions.

The following are the authors' abstracts of papers presented.

Electron microscopic observations on human skin. By G. Causey. *Royal College of Surgeons, London*

The structure of the human skin, viewed by the electron microscope in thin section, was the subject of a short report by Pease (*Amer. J. Anat.* 1951, **89**, 469–497). A further examination of thin skin from the abdominal wall has been made. Attention will be drawn to the keratohyalin granules in the stratum granulosum and their relation to the fibrillary cytoplasm in the deeper layers of this stratum. There is a gradual transition to the bundles of tonofibrils in the stratum spinosum. The distribution of collagen in the dermis was illustrated and contrasted with the distribution of collagen and elastic tissue bundles in the rare condition of Pseudoxanthoma elasticum.

Electron microscopy of the high level clear cell (Langerhans cell) in normal and vitiliginous human epidermis. By M. S. Birbeck, A. S. Breathnach and J. Everall. *Chester Beatty Research Institute, and St Mary's Hospital Medical School, London*

Features of cells of characteristic appearance situated at supra-basal levels of the epidermis in normal and vitiliginous skin are described. Their cytology as revealed by the electron microscope is in many ways similar to that of a basal melanocyte, but differs from that of a keratinized epidermal cell. The cells contain neither melanin nor pre-melanin granules, but they do contain a characteristic disc-shaped granule, smaller than a melanin granule, and the nuclear membrane is convoluted. Such cells appear to be more numerous in vitiliginous than in normal epidermis.

It is concluded that the cells described represent the high level 'clear' and aureophilic cells of light microscopy. These latter are commonly thought to be effete melanocytes on the way towards exfoliation at the surface of the epidermis. The bearing of the present observations on this hypothesis was discussed.

Observations on the histology and histochemistry of the hairy and non-hairy parts of human skin. By M. Singh. *University of Aberdeen*

Human skin does not show alcohol-fast metachromasia when stained with aqueous or alcoholic solutions of toluidine blue or azure A by normal procedures.

If sections of skin still in the paraffin ribbon are first incubated at 37° C. with 0·2 %

pepsin solution and thereafter stained with these dyes, then alcohol-fast metachromasia can be demonstrated in antemortem and postmortem material from the hairy part of the skin, sole and palm, when fixed within 12 hr. The bald part of the scalp, however, shows no metachromasia when treated in the same way for the same length of time.

If fixation is delayed for more than 36 hr., then both the bald and hairy parts of the skin show metachromasia. The metachromasia is labile to testicular hyaluronidase and may be due to chondroitin sulphate B or C or both.

The depth of the papillary layer is greatly reduced in the bald area of the scalp and the basement membrane, and collagen bundles are much thicker than in skin from elsewhere.

It is claimed from these findings that the acid mucopolysaccharides (chrondroitin sulphates) of the ground substance in the papillary layer of the bald scalp are in a higher state of polymerization or in a firmer combination with the carbohydrate-protein complex than elsewhere.

The ultrastructure of the mouse prostate gland. By A. A. Barton. *Royal College of Surgeons, London*

The normal gland has a number of acini, each of which consists of a layer of cells interdigitating with one another, surrounding a central lumen. At the base of these cells are a number of nuclei belonging to the basal cells, which are seen with the electron microscope to possess processes of cytoplasm forming an almost continuous layer separating the cells bounding the lumen from the smooth muscle cells, blood vessels and fibrous tissue which form the outer boundary of the acinus.

The cells which surround the lumen are the secretory cells. Both in the normal prostate gland and, more particularly, in glands stimulated by testosterone, a secretory cycle has been recognized, culminating in the extension of the lumenal side of the cell into the cavity to form the prostatic secretion. With this process the cells become smaller, the lumen distended, the smooth muscle cells surrounding it more tenuous and the convolution of their outer borders less obvious. The changes are almost identical to those seen in the uterus stimulated with oestrogens.

A comparison of the cerebral vascular system in cat, dog and man. By B. D. Wyke and D. H. Tompsett. *Royal College of Surgeons, London*

The differences between the cerebal circulation in man and the cerebral circulation in laboratory animals have been of considerable interest both to anatomists and physiologists, particularly in relation to the transfer of experimental findings on laboratory animals to man.

By using injection of plastics developed by one of us (D. H. T.), it has been possible to get corrosion casts of the cerebral arterial tree, including filling of the vessels down to small arterioles. The gross differences between the circulation in the three cases was discussed and additional evidence with regard to anastomosis of small vessels brought forward. Emphasis was placed on the differing contribution from internal and external carotid sources.

A case of conjoint twins. By R. M. Livingston. *Royal College of Surgeons, London*

The incidence of conjoint twinning is difficult to assess, and it may be that it is more common than has been thought. It is still a rarity, however, especially when the infants survive and the question of separation may be considered. In the case to be described the children lived for 5 weeks. They were born to a native family in the Kano district ofNorthern Nigeria and came under the care of Mr John Craddock, F.R.C.S., at Kano. The twins separated at the Hammersmith Hospital in 1953 by Professor Ian Aird and his team were from the same district. The deformity was of the thoracopagus type. Preliminary investigations suggested that there was a bridge of liver between the two and a connexion between the two alimentary canals. It was thought, however, that the hearts were separate, as the pulses could be made to differ. The post-mortem findings may be considered in two categories—one concerned with the twinning and the other with a series of rarely described abnormalities of the large vessels of the thorax, present, but different, in each infant. These findings were described and their embryological background reviewed.

The 'Subclavian Shelf' and its possible relation to coarctation of the aorta. By D. B. Moffat, *University College, Cardiff*

The origin of the left subclavian artery has been studied in eighty-five rats of ages varying from 3 days before birth to 3 weeks after birth: ten human foetuses were also studied. In the rat, at about the time of birth, the origin of the left subclavian artery becomes slightly constricted by a 'shelf' of tissue which projects into its lumen from the distal margin of the orifice. This becomes larger with increasing age and reaches its largest size relative to the vessels at about 7–8 days after birth; at this time it is often large enough to produce some narrowing of the aorta. In the early stages, the 'shelf' is formed by an infolding of the whole vessel wall and appears to be due to the migration of the subclavian artery relative to the aorta. Later, the 'shelf' takes the form of a pronounced thickening in the media. A similar 'shelf' is found in the human, but it appears several months before birth. It is suggested that an exaggeration of this 'shelf' may be a cause of the 'adult' form of coarctation of the aorta.

On the presence of nerve fibres in the pars tuberalis of ungulates after pituitary stalk section.* By Elisabeth Beck and P. M. Daniel. *Maudsley Hospital, London*

The pituitary stalk was cut in eight sheep and twelve goats, an impenetrable barrier placed between the hypothalamus and the pituitary fossa. Survival times ranged from 4 days to 15 months. In serial sections through each hypothalamus and six controls, no nerve fibres (Bielschowsky) or CAH-positive deposits (chrome-alum-haematoxylin and alcian blue) were found in the pars tuberalis of the controls. In the experimental animals, from 21 days post-operatively onwards, an increasing number of nerve fibres, many showing CAH-positive beading, were seen leaving the neural tissue and ramifying amongst the glandular cells, where CAH-positive material accumulated around blood vessels. Soon after operation mitotic figures were found in the pars tuberalis which had hypertrophied in long survivals.

There was loss of nerve cells in the supraoptic and paraventricular nuclei, but some of the surviving cells contained neurosecretory granules in the perikaryon and axon. It seems likely, therefore, that those neurons which survive, or recover from severance of their axons, continue to elaborate a neurosecretory substance which is carried down their axons to be stored not only in the regenerated neural portion of the stump (Beck, E. & Daniel, P. M., 1959, *J. Physiol.* 146, 22*P*) but also in the pars tuberalis. We have seen a similar picture in man and monkey.

* This work was assisted by a grant from the Nuffield Foundation.

The innervation of avian striated muscle. By S. H. Shehata and Ruth E. M. Bowden. *Royal Free Hospital School of Medicine, London*

The innervation of post-vertebral, pectoral, and leg muscles has been studied in forty seven chicks of 2- to 94-days-old. The gross pattern of intramuscular nerve branching and the distribution of motor end-plates were studied and correlated. The subneural apparatus, as demonstrated by the Köelle and Friedenwald stain (*Proc. Soc. Exp. Biol., N.Y.*, 1949, **70**, 617–622), is simple in young animals and becomes complex with age. Both simple and elaborate motor endings were found on red and white muscle fibres.

Muscle spindles were found in semispinalis, tibialis anterior, and gastrocnemius muscles. They appeared to be less numerous in pectoralis major and secundus, and were not found in biventer. The number of intrafusal muscle fibres varies between 1–6. Multiple motor endings derived from either a single or from apparently different axons were seen in the polar region. The nerve endings in the equatorial region were mainly of the flower-spray type, and annulospiral endings were rare.

The tendon organs were arranged in groups, and no Pacinian corpuscles were found.

Tissue culture studies of lymphoid tissue after skin homografting in rabbits.

By J. A. SHARP and R. G. BURWELL. *University of Leeds*

Unsuccessful attempts have been made in the past to reproduce the homograft reaction *in vitro* (Medawar, P. B., 1948, *Quart. J. Micr. Sci.* **89**, 239–253; Scothorne, R. J. & Nagy, I., 1960, *J. Anat., Lond.*, **94**, 454.) Using a simple tissue culture chamber, we have studied the effect of growing explants of spleen from the donor animal with either reactive lymph node (R.L.N.) which had drained a skin homograft recently applied to the ear, or inactive lymph node (I.L.N.) from the ungrafted side. Two series of chambers were set up, one containing two donor spleen explants with six explants of R.L.N., the other containing two donor spleen explants with six explants of I.L.N. Additional cultures of donor spleen without lymph nodal tissue were studied.

Observations have been made on the rates of growth of the donor spleen explants under the different conditions. The results were discussed in the light of previous work on the homograft reaction *in vitro*. Differences were also described in the cellular outgrowths from both the R.L.N. and I.L.N. cultures.

A study of the first regional lymph nodes draining homografts of red bone-marrow, marrow-free cancellous bone and certain stored bones.

By R. G. BURWELL. *University of Leeds*

In a previous communication (Burwell, R. G., 1960, *J. Anat., Lond.* **94**, 455), the response of the first regional lymph node to homografts and autografts of fresh cancellous bone was reported.

An attempt has been made to utilize the changes occurring in the lymph node draining the homograft to assess, not only the antigenicity of two *components of fresh bone*, but also the relative antigenicity of certain *stored bones* which are, or have been, used in clinical bone grafting.

The homografts were inserted into the subcutaneous tissue of one ear in fifty-four rabbits, and the first regional (parotid) lymph node removed for examination at 5 and 8 days after operation. The immune response to the components of *fresh* cancellous bone was investigated after the insertion of constant weights of red bone-marrow and marrow-free iliac cancellous bone. The *stored* bone used included freeze-dried, boiled, frozen (–20° C.), irradiated and merthiolate-preserved cancellous bone.

The weight increase of the lymph nodes on the grafted side was greatest in those animals receiving bone-marrow. Marrow-free cancellous bone and all the stored bones produced little increase in lymph node weight, comparable with that produced by autografts of cancellous bone.

The 'large lymphoid cell' response to the homotransplants of bone-marrow was marked, but with certain exceptions, the marrow-free bone and the stored bones produced little change in the 'large lymphoid cell' content of the nodes.

The findings were discussed in the light of present knowledge concerning the transplantation immunity of bone.

Studies in foetal blood formation. I. The changes in the peripheral blood. By D. Brynmor Thomas, P. M. Russell and J. M. Yoffey. *University of Bristol*

The blood has been studied in thirty healthy foetuses and newborn infants from the 55 mm. crown–rump length stage onwards. The erythrocyte count rises from $1{\cdot}36 \times 10^6$/mm.^{3} in the earliest foetus to values ranging about 4×10^6 at full term, while the haemoglobin concentration rises from 7 g./100 ml. to values ranging about 15 g./100 ml. Until the 200 mm. stage is reached the increase in red cell count and haemoglobin concentration occurs very rapidly but thereafter proceeds more slowly. The nucleated red cells fell from 54,200/mm.^{3} in the blood of the 55 mm. foetus to 1200/mm.^{3} at 177 mm. and subsequently form an insignificant proportion of the circulating red cells. The very marked anisocytosis displayed by the red cells of the earliest foetuses examined becomes much less marked as development proceeds and is relatively inconspicuous by the 96 mm. stage. The mean corpuscular haemoglobin is raised during foetal life, averaging 47 in a series of fifteen foetuses ranging from 55 to 230 mm.; as the mean corpuscular volume is proportionately increased, however, the mean corpuscular haemoglobin concentration approximates to the normal adult range. At term the mean corpuscular haemoglobin and mean corpuscular volume are still raised above the normal adult range but less so than in the early stages of development.

Throughout the period covered, cells of the myeloid series were present in the blood, rising steadily in number during development from 570/mm.^{3} at the 55 mm. stage. Small lymphocytes, which were first seen in the blood of an 85 mm. foetus increased in a similar fashion. Many of these small lymphocytes are of the 'transitional' type previously described in guinea-pig bone-marrow (Yoffey, J. M., 1957, *Brookhaven Symposia in Biology*, **10**, 1).

Studies in foetal blood formation. II. Observations on the bone-marrow. By J. M. Yoffey, D. Brynmor Thomas and P. M. Russell. *University of Bristol*

The bone-marrow has been studied in fourteen human foetuses ranging in size from 96 to 228 mm. The femoral marrow has been examined both in air-dried smears upon which differential counts were performed after staining with MacNeal's tetrachrome stain, and in sections of the marrow *in situ* in decalcified bone. Unfortunately it was not found possible to perform full quantitative estimations, as had previously been done in the guinea-pig (Yoffey, J. M. 1957, *Brookhaven Symposia in Biology*, **10**, 1), since the arrangement of cancellous bone in the marrow cavity made it impossible to obtain a marrow plug. The differential counts reveal a surprisingly constant distribution of the main cell groups. At 96 mm. the cells were: myeloid 27 %, erythroid 14 %, lymphocytes 27 %, damaged 30 %, miscellaneous 3 %. At 228 mm. the cells were: myeloid 25 %, erythroid 10 %, lymphocytes 45 %, damaged 14 %, miscellaneous 4 %. There are never any lymphoid nodules in the marrow, which nevertheless possesses 3 to 4 times as many lymphocytes as adult marrow. There is a high proportion of 'transitional' cells in foetal marrow. The sinusoids are large and dilated. The circulation is a closed one.

Studies in foetal blood formation. III. Hepatic haemopoiesis. By D. Brynmor Thomas, P. M. Russell, and J. M. Yoffey. *University of Bristol*

The liver has been studied in twenty-two fresh, healthy, human foetuses both in dry smears, prepared from cell suspensions, and in sections. Red cell formation has been evident at all the stages examined, from 45 to 228 mm. but little if any granulopoiesis or lymphocytopoiesis has been observed. Granulocytes are found occasionally in the circulating blood contained in the sinusoids, as also are lymphocytes. The red cells appear to develop as islands in the liver cords, and our observations lead us to support the view of Toldt & Zuckerkandl (*S.B.S. Akad. Wiss. Wien*, 1875, 72, Abt. 3, 241), that the haemocytoblast in this situation is a modified liver cell. We can find no evidence to support the widely accepted view of Maximow (*Arch. mikr. Anat.* 1909, 73, 444) that hepatic erythropoiesis is attributable to proliferating mesenchymal cells.

The effect of insulin in the early chick embryo. By Patricia Barron and J. McKenzie. *University of Aberdeen*

Twenty-four-hour chick embryos, explanted according to the New and Spratt techniques were treated with varying concentrations of therapeutic insulin. Irrespective of the mode of application of the hormone, the results, after a further 24 hr. culture *in vitro* were the same, viz. marked inhibition of the central nervous system, the neural tube remaining open to a greater or lesser degree. The heart developed normally in most cases and only occasionally did the somites show slight anomalies. Cellular necrosis was absent in the affected regions except for occasional lysis around the primitive streak. Control embryos showed no defects.

Theories on the possible mode of action of insulin in this context were reviewed and the application of these results to the aetiology of abnormalities of the central nervous system, such as anencephaly, were discussed.

Studies in head morphogenesis: development of isolated presumptive trabecular tissue in the chick embryo. By P. H. S. Silver. *Middlesex Hospital Medical School, London*

Phylogenetically, the primitive form of the trabeculae cranii is the platitrabic, in which they remain separated throughout almost their entire length. The later development of a midline trabecula communis (tropitrabism) is associated in birds with the acquisition of large eyes. The question may be raised as to whether the eyes provide a possible causal mechanism capable of influencing trabecular pattern in ontogeny. Removal of both eyes does not appear to influence trabecular pattern, but further evidence against such a causal mechanism has been obtained by removing the primitive trabecular tissue at stages of ten to twelve somites from embryos from which the presumptive eye tissue had previously been removed at the four-somite stage. The fragment was then grown in the intraembryonic coelom of another host embryo. Twenty such experiments have been performed. A coherent organized graft develops, which bears an unmistakable resemblance to the upper beak, although naturally the nasal cavities are completely absent. The cartilaginous trabecula communis develops along with membrane bone identifiable as the premaxilla. The conclusion seems to be that although the eyes exert a considerable influence during the stage of cartilage growth, they play no essential role during the more fundamental stages of determination and differentiation.

The development of the sclera and choroid in staged chick embryos. By R. O'Rahilly. *Wayne State University*

A PAS-positive membrane is found on the external aspect of the external lamina of the optic cup by stage-16 and it is covered by a capillary layer. At stage-24 a mesodermal condensation for the sclera is present external to the strata mentioned above and, at stage-28, cartilage is observed in the ablenticular part of the eye. On the appearance of ciliary processes at stage-29, the greater extent of the vascular layer may be termed the choriocapillaris. The scleral cartilage is distinctly PAS-positive from about stage-30 onwards. At stage-35 the fibrous portion of the sclera is clearly continuous with the substantia propia of the cornea. By stage-36 the choroid comprises a vascular lamina, a choriocapillaris, and a basal lamina. At the level of the ora serrata, about fourteen scleral ossicles appear external to the fibrous sclera at stage-37. Apparently they are related to the overlying conjunctival papillae (Murray, P. D. F., 1943, *J. Anat., Lond.*, 77, 225–240.; Hale, L. J., 1956, *Quart. J. Micr. Sci.* 97, 333–353), which appear from stage-30 to stage-34. The ossicles become curved and begin to be overlapped by the underlying scleral cartilage at stage-38. They are visible grossly by means of alizarin red S at stage-39. The initial appearance of pigment in the choroid is variable; one specimen was markedly pigmented at stage-40, whereas some specimens show little or no pigment even in the adult.

The giant cell trophoblast of the lesser bush baby (*Galago senegalensis senegalensis*). By H. Butler. *University of Khartoum*

An 0·5 mm. diameter blastocyst has an area of giant cell trophoblast (G.C.T.) measuring 500 by 100 μ and 50 μ thick. G.C.T. is in contact with maternal stroma. G.C.T. expands in area, up to 4·5 by 1·0 mm. in the 5·0 mm. diameter blastocyst, but does *not invade any deeper*. When the blastocyst reaches 7·0 mm. diameter the G.C.T. has almost disappeared and uterine epithelium re-covers the denuded area. In the 4·5 mm. blastocyst the small cell trophoblast shows circumscribed thickening over the mouths of the uterine glands, the pro-chorionic vesicles. The latter increase in size and number as the G.C.T. disappears. At the eighteen-somite stage the uterus is completely covered by epithelium and only ghost-like remnants of G.C.T. remain. The numerous pro-chorionic vesicles are now 0·25 mm. or more in diameter.

The 'mesenchymatous' endoderm of the lesser bush baby (*Galago senegalensis senegalensis*). By H. Butler. *University of Khartoum*

Gerard (*Bull. Acad. roy. Med. Belg.* 1932, **11**, 662–682) described the early endoderm of *Galago demidovii* as forming a 'mesenchymatous' mass surrounding a small cavity and only later becoming a typical epithelial layer. In all stages examined in the present material there is a large yolk sac cavity and the bulk of the extra-embryonic endoderm is a single layer of epithelial cells. From the 4·5 mm. diameter blastocyst onwards a localized area of 'mesenchymatous' endoderm is found at the tubal pole of the blastocyst. This area of modified endoderm is not related to the embryonic area and the eighteen-somite stage clearly shows that it is not related to the extra-embryonic mesoderm. It is avascular, contains amorphous eosinophilic deposits and the overlying small-cell trophoblast is thickened and folded. It always overlies the mouth of the Fallopian tube.

Embryonic tissue homografts in rats. By J. Wilson Clarke (introduced by J. J. Pritchard). *Queen's University, Belfast*

Homografts of placenta, splanchnopleure and limb-buds from 14-day outbred rat embryos were transplanted to the rectus abdominis muscles of their mothers, unrelated males and unrelated pregnant females.

A few placental trophoblast cells survived for a week or two in all hosts. Splanchnopleuric grafts were rapidly destroyed in males and unrelated females, but in mothers the grafts formed endodermal cysts with goblet cells which perisisted up to 40 days. Cartilage models differentiated from the mesenchyme of the grafted limb-buds within 4 days in all hosts. In the mothers primary and secondary endochondral ossification resulted in the formation of well-organized ossicles. In other hosts ossicles did not develop: the cartilage evoked a vigorous lymphocytic reaction, died and was eroded by giant cells. Some new bone, presumably of host origin, formed in relation to the eroded cartilage. Stratified squamous epithelial cysts also developed, surviving indefinitely in the mothers but being destroyed within 20 days in the other hosts.

It would appear that the mother has relative immunological tolerance towards the tissues of her offspring *in utero*.

The development of the sternomastoid and trapezius in man. By J. McKenzie. *Department of Embryology, Carnegie Institution of Washington, Baltimore, U.S.A., and the University of Aberdeen*

In a previous communication (*J. Anat., Lond.*, 1954, 88, 584) upon the comparative anatomy of the sternomastoid and trapezius and their appearance in a 9 mm. human embryo, it was suggested that these muscles, contrary to the accepted teaching, have a myotomic element in their composition.

Serial sections of thirty-two human embryos ranging from 4·85 to 14 mm. (Streeter's groups XIV to XVIII), examined with special reference to the development of the accessory and neighbouring musculature, show that the sternomastoid and trapezius premuscle masses are so closely associated with myotome material that it is no longer possible to maintain that they are entirely of branchial origin. It appears now that the deep part of the human sternomastoid corresponds to the omocervicalis of other mammals and that the trapezius extends dorsally and caudally by increments from the same premuscle material which gives rise to the rhomboidei layer.

The findings afford an explanation of the dual innervation of the sternomastoid and trapezius muscles and of the conflicting results of experimental procedures in different animals.

Further studies of the sensitivity of erythrocytes to nitrites: the effects of age, pregnancy and malignant disease. By W. K. Metcalf. *University of Bristol*

In November last year I presented a paper to this Society describing an investigation into the differing sensitivity of red cells to nitrites. To enable a more extensive survey to be undertaken, a more rapid and convenient technique has been devised to measure this sensitivity.

The effect of age has been re-investigated and the previous results confirmed. In an attempt to elucidate this phenomenon more than fifty pregnant women have so far been tested and all show the characteristic increased sensitivity of juveniles. The changeover has certainly occurred by the 7th week of pregnancy, and returns to normal within a few hours of delivery.

More than thirty-five patients with a diagnosis of carcinoma of various organs have also been investigated and some 90 % show increased sensitivity of their red cells.

The possible causes and significance of these findings were discussed.

Observations on the form and orientation of epiphyseal cartilages. By J. W. Smith. *University of St Andrews*

Each epiphyseal cartilage tends to have its own characteristic shape and orientation. A radiograph of an immature calcaneum in which the compression and tensile trabeculae are readily visible, suggests that, in that bone, the disposition of the epiphyseal cartilage is closely related to the stress distribution. In order to study this relationship in other epiphyseal cartilages in the lower limb, stress analyses of the femur and tibia were carried out by the photo-elastic method.

It has been demonstrated that in the regions examined, epiphyseal cartilages are, in large measure, orientated so that they lie parallel to the direction of one of the principal stresses: usually this is the tensile stress, but in some situations it is the compression stress.

The mechanical advantage of this orientation of the epiphyseal cartilage is that shear stress between the metaphysis and the epiphysis is reduced to a minimum.

Cross-sectional area of the end-surfaces of cat vertebrae. By R. N. SMITH, D. G. LEE and A. S. KING. *The University of Bristol*

Measurements of human vertebrae show an increase in the area of cross-section of their end-surfaces from the second cervical to the last lumbar. This has been claimed to be related to the upright posture of man. During the course of a previous communication to this Society (*J. Anat., Lond.*, 1959, **93**, 590) it was shown that a similar increase occurred in the dog and it was suggested that this might be a general mammalian characteristic as opposed to a bipedal one. This area has been examined in the cat and from measurements of twenty specimens it is found that there is also an increase along the vertebral column of this species, but only in the thoraco-lumbar region. In the cervical region the cross-sectional area shows a decrease in succeeding vertebrae. A progressive increase of cross-sectional area of the end-surface of vertebrae is therefore not a constant mammalian characteristic, nor is it restricted to those with bipedal gait.

The development of the os genitale of *Sciurus carolinensis* By A. YOUNG. *University of Glasgow*

The presence of an os genitale has been reported from a wide variety of adult mammals. It is usually smaller and less constantly present in the female of those species in which it occurs. It probably has some taxonomic value in determining species relationships. The os genitale of the Grey Squirrel (*S. carolinensis leucotis*) presents some interesting features of development and form in both sexes. Ossification starts within a few days of birth by the deposition of osteoid in the centre of a mass of dense precartilaginous tissue. Some time later, true cartilage appears at the base of the bony rod and some of the distal precartilaginous tissue becomes cartilage also. The distal end in the male assumes a bizarre shovel shape which is asymmetrical in relation to the urethra; in the female the bone is not quite so complex but it still is asymmetrical in relation to the glans clitoridis. In both sexes distal flanges lie remarkably close to the epidermis of the glands.

The effect of distension of the urinary bladder on the lining epithelium and on its histochemical reaction for alkaline phosphatase. By B. F. MARTIN. *University of Sheffield*

A study was made on guinea-pigs (mainly females) following both acute and chronic distension of the bladder. Acute distension was produced by injecting saline into the bladder through a glass catheter and chronic distension by ligating the bladder neck for varying periods up to 24 hr. The bladders were fixed in the distended state in 80 % alcohol. The purpose of the study was to determine the mechanical effect of distension on the lining epithelium, the effect on the alkaline phosphatase reaction and also to ascertain whether distension would stimulate the cells of this epithelium to divide.

After distension, the epithelial cell layers are reduced in number from 5, to 2 or 3, and the cells are elongated parallel to the surface. The reaction for alkaline phosphatase, which is located in the tunica propria and in the apical cytoplasm of the epithelial cells, is markedly reduced after both acute and chronic distension, but returns to normal if the bladder is emptied. Some indication of the cell movement in distension is given.

After chronic distension, a number of specimens showed the presence of mitotic figures in the epithelium, even after as little as 6 hr. distension.

Melanocyte pattern of regenerated human epidermis. By A. S. Breathnach. *St Mary's Hospital Medical School, London*

The melanocyte pattern of the epidermis covering scars 2 and 3 weeks old in a human subject was investigated using various techniques. Material examined included: (1) 'Split-skin' preparations incubated in Dopa, or stained supra-vitally with methylene blue 1/2000; (2) transverse sections of skin incubated entire in Dopa and post-impregnated with Masson's silver nitrate solution; (3) transverse sections of skin treated with gold chloride (Gairn's).

Potentially active melanocytes in reduced numbers (as compared with adjacent normal skin) were found in epidermis covering peripheral areas of the scars, but none was present in more centrally-lying areas. Examination of the supravitally stained material indicated that this latter finding is due, not to complete inactivity of melanocytes actually present, but to their total absence. There would appear therefore to be a dissociation between melanocytes and Malpighian cells in the epidermis covering the central areas of the scars. It is argued that the appearances presented seem to be consistent with the postulate that melanocytes are capable of migrating into a preformed sheet of Malpighian cells.

Alkaline and acid phosphatases in the salt glands of birds. By R. J. Scothorne. *University of Glasgow*

Acid and alkaline phosphatases have been studied histochemically in the salt-secreting (nasal) glands of the duck and the herring gull.

Alkaline phosphatase is sparse in the glandular epithelium of resting, active and recovering glands of the duck, and absent from the intralobular capillaries. In the inactive gland of the gull it is absent from the glandular epithelium, but present in the intralobular capillaries.

Acid phosphatase is abundant in the epithelium of the ducts and of secretory tubules in inactive, active and recovering glands in the duck and in inactive glands of the gull. It is localized principally in the supranuclear portion of the cell.

These findings were discussed in relation to the distribution of phosphatase in a number of other cells which are thought to be concerned, as the nasal gland cell certainly is, with active ion transport.

The fine structure of the nasal gland in the Aylesbury duck. By R. J. Scothorne and A. D. Hally. *University of Glasgow*

Previous histological and physiological studies by one of us (R. J. S.) revealed that the nasal gland of the duck was capable of secreting a strongly hypertonic solution of practically pure saline. The present study describes the fine structure of this compound tubular gland.

The adjacent surfaces of the secretory cells are separated from one another over most of their extent by prominent intercellular canaliculi which are open basally. The canaliculi appear not to communicate with the lumen of the tubule, but are occluded by terminal bars. A few small microvilli project from the apical surface into the lumen of the tubule. The cells show extensive basal infolding of the cell membrane, such as is seen in other cells concerned with the transport of ions and water (Pease, D. L., 1956, *J. biophys. biochem. Cytol.* 2, 203–208). The abundant mitochondria contain numerous cristae and a dense ground substance. The granular endoplasmic reticulum is scanty. The Golgi complex is not prominent, and consists of groups of membranes and vesicles lying lateral or apical to the nucleus. Vacuole-containing bodies are present.

The above findings were discussed in relation to other cell types of similar function.

Comparative anatomy of the respiratory pathways in the fowl, pigeon and duck.

By A. R. Akester. *University of Cambridge*

Latex casts of the respiratory system in the fowl, pigeon and duck were used to study the distribution of primary, secondary and tertiary bronchi in the lungs, their connexions with air sacs and pneumatic bones, and the relationship between the air sacs and viscera in the body cavity.

Each lung contains a primary bronchus, anterior and posterior groups of secondary bronchi which are connected together by many tertiary-bronchial arcades, and innumerable air capillaries which radiate out from recesses in the walls of tertiary bronchi.

There are four anterior dorsal secondary bronchi, eight or nine posterior dorsal secondary bronchi and seven or eight posterior ventral secondary bronchi.

The classical 'vestibule' in the primary bronchus was not found in any cast. Direct connexion between the primary bronchus and the posterior thoracic air sac, in all cases, was by the second posterior ventral secondary bronchus and not by a bifurcation in the primary bronchus.

In the adult lung the so-called 'recurrent bronchi' were found to be indistinguishable from tertiary bronchi and it is suggested that this concept of an alternative return route for air from the sacs to the lungs may be misleading.

The structure of the lungs in the fowl, pigeon and duck were found to be very similar. The most significant difference was the diameter of the tertiary bronchi. The strong flying duck and pigeon had about 4 times as many tertiary bronchi (diameter about 0·5 mm.) per unit volume of lung as were present in the relatively flightless fowl (tertiary bronchi diameter about 1 mm.).

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